REFERENCE MANUAL

FOREIGN ANIMAL DISEASE COURSES

Plum Island Animal Disease Center

UNITED STATES DEPARTMENT OF AGRICULTURE
AGRICULTURAL RESEARCH SERVICE
NORTHEASTERN REGION
PLUM ISLAND ANIMAL DISEASE CENTER
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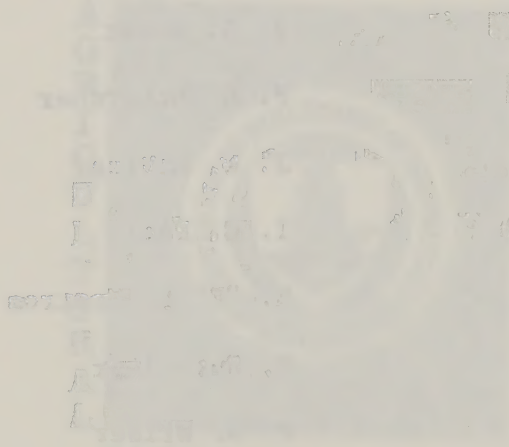

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REFERENCE MANUAL

FOREIGN ANIMAL DISEASE COURSES

FORWARD

This reference manual provides up-to-date background information on those foreign animal diseases which are demonstrated in PIADC courses. The major objective of these courses is to give field diagnosticians experience in the clinical aspects of selected exotic diseases (as well as a few similar domestic diseases). Most of your time will be spent participating in actual disease exercises and demonstrations. During the short time you are at PIADC you may not have time to avail yourself of our excellent library or to read lengthy reviews. There is a vast and scattered literature on many diseases, such as foot-and-mouth disease; on others, such as swine vesicular disease, little has been written and the few papers that exist might be difficult for you to look up in the field.

The reviews of each disease have been prepared and reviewed by the PIADC faculty to provide you with succint and cogent data on the diseases you will meet in the laboratory.

You may find the Reference Manual of value later, also. For field use PIADC has prepared a Pocket Handbook on diseases exotic to most of the Americas. This book has been scaled down to pocket size, but is so designed that it still contains a great deal of memory jogging information, including more than 150 color illustrations of the main clinical features of 10 major exotic diseases. These may be readily studied in a microfiche reader, with a 10X hand lens or even projected by the TAYLOR-MERCHANT microfice projector* and similar inexpensive devices. The color illustrations and the short descriptions may be valuable in emergency situations.

If lesions other than those illustrated are seen in the demonstrations or in the field, the Pocket Handbook will be updated to include these in the future. As new data are accumulated both the Reference Manual and Pocket Handbook will be revised and the new editions made available.

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*Taylor-Merchant Corporation, New York, N.Y. 10036. This is for information only and does not imply endorsement.

There are numerous other diseases foreign to the Americas which cannot be included in the courses within the time allotted. You are urged to obtain a copy of FOREIGN ANIMAL DISEASES--U.S. ANIMAL HEALTH ASSOCIATION ("The Gray Book", circa 1964)* for information of approximately the same scope on a much larger number of exotic diseases. A new edition of this book approaches completion.

For your convenience an outline of procedures on the collection of specimens for the laboratory has been prepared. Appended to this is a complete excerpt of data from EMERGENCY ANIMAL DISEASE ERADICATION GUIDE ("The Red Book") from the Animal and Plant Health Inspection Service (APHIS) on this subject.

You may have other interests. In each class there have been those interested in some aspect of research or the laboratory tests used to make or confirm diagnoses. We have microfiche giving detailed protocols for the large scale production of bovine serum and the preparation of reagents and performance of several standard laboratory tests,

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*U.S. Animal Health Association, 1444 East Main St., Richmond, Va., 23219

Copies of these and reprints or reviews of a more specialized nature may be requested from the Director, PIADC.

AGENT SAFETY INSTRUCTIONS *

Shower Exits

1. All persons leaving an area will completely disrobe in the interior clothes change room.
2. Each person should then blow his nose in a paper towel and attempt to clear his throat and expectorate. The purpose of this action is to attempt to remove from the upper respiratory tract the mucous materials containing virus particles which may have been inhaled while in the laboratory.
3. The fingernails should then be carefully cleaned, using the fingernail files hanging by the wash basins.
4. The hands should be scrubbed with a brush, paying particular attention to cleaning the sides of the fingernails.
5. Each person should then enter the shower and spend at least one minute soaping the entire body, including the hair, with officially approved soap.
6. At least one minute should be spent in completely washing off the soapy water.
7. After completion of the decontaminating shower, each person should step out of the shower stall into the clean locker room to dry. The towel should be placed in the laundry hamper after use.
8. Personal clothing may then be donned and an exit made from the laboratory.
9. Allow sufficient time for decontamination procedures. The act of hurrying through a shower without following all instructions properly is considered a first-degree agent safety violation.

*Prepared by J. L. Hyde

AFRICAN HORSE SICKNESS *

I. Identification of disease:

A. Definition.--African horse sickness (AHS), also termed perdesietke and pestis equorum, is an insect-borne disease of equidea, caused by a virus of 9 immunological types. It occurs as an acute or subacute disease, and is highly fatal in a susceptible equine population.

B. Etiology.--The virus particle is small, measuring 70-80 nm in diameter and is estimated to have 92 rod-shaped subunits radiating from a spherical body. The virulent virus can be maintained in OGC solution at 4° C for several years, but it is destroyed in 2 weeks at 37° C. The virus is destroyed by treatment with acetic acid (pH 3 and lower), and its infectivity is destroyed in 5 minutes at 70° C.

The virus was adapted to baby mice by intracerebral inoculation. Mortality in mice increases upon further passages, with a subsequent decrease in virulence of the virus for horses. Although the virus loses its pathogenicity for equidae, it does not lose its antigenic and immunizing capabilities for these species.

C. History.--The disease was endemic in Africa and apparently caused great mortality in equidae which were brought by the early settlers. Early research with the virus was slow until Alexander (1933-1935) adapted the virus to mice by intracerebral passage. The disease was restricted to Africa until it made its appearance in Middle Eastern countries. In 1966 it spread to North Africa and the southern tip of Spain. The disease there was eradicated by 1969 as a result of vaccination and other measures.

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*Prepared by A. H. Dardiri

II. Signs:

A. Clinical features.--To the clinician observing an early case of AHS, the most obvious signs are depression and high fever. However, the signs of the disease vary, depending on the form in which it manifests itself.

The pulmonary form is acute and may be of quite sudden onset, marked by respiratory involvement and fever. Coughing spasms occur, and large quantities of frothy fluid may be discharged from the nostrils. The head and neck are extended, the ears droop, and severe sweating occurs. Finally, the animal lies down, nearly choking. Until the terminal stages, the animal appears hungry and attempts to eat. This form of the disease is highly fatal.

The cardiac form is characterized by fever and a slower course. Swelling of the head, neck, and chest are typical manifestations of this form. Edema of the supraorbital fossea, eye lids, and lips is common. Recovery is more common with this form.

A mixture of the pulmonary and cardiac forms may also occur, with either one predominating initially; the mixed forms are less readily diagnosed until necropsy examination is made.

Mild cases may also occur with the only indications being a rise in temperature, rapid pulse, and difficult breathing. This form occurs most often with the less virulent viruses or where some degree of immunity is present. Slight symptoms of this type may be seen following vaccination.

B. Incubation period.--The incubation period is less than 9 days under natural conditions. Experimentally, the incubation period varies from 2-21 days. Usually it is about 14 days.

III. Pathologic changes:

A. Postmortem lesions.--The lesions vary in accordance with the form of disease. With the pulmonary form, the most conspicuous lesions are edema of the lungs and hydrothorax, the lungs remaining fully distended. The subpleural and interlobular tissues are heavily infiltrated with yellowish gelatinous exudate. The bronchi, trachea, pharynx, and nostrils are filled with froth and fluids. The stomach contains a viscid mucous mixed with food. Also, the mucosa of the fundus is reddened and edematous.

In the cardiac form, the outstanding lesions are gelatinous exudates in the subcutaneous, subfacial, and intramuscular tissues and lymph nodes. A massive hydropericardium is always seen and hemorrhages and ecchymosis are found on the epicardial or endocardial surfaces, or bordering the coronary vessels.

As in the pulmonary form, acites may be found, and the mesentery glands may be swollen. Edema of the lungs is slight. Stomach lesions are the same as in the pulmonary form.

In the mixed form of AHS, lesions common to both the lung and cardiac forms are found.

B. Microlesions.--The histopathological changes in the equine organs are the result of increased permeability of the capillary walls and impairment of the circulatory system.

The lungs show serous infiltration of the intralobular tissue with distention of the alveoli and congestion of the capillaries.

The central veins of the liver are distended, the interacinous tissue contains erythrocytes and blood pigment, while the parenchymatous cells exhibit fatty degeneration.

Various degrees of round cell infiltration can be seen in the cortex of the kidneys.

The spleen is congested and shows hemorrhagic extravassation into the pulp.

Variable degrees of congestion are present in the gastric and intestinal mucosae, and cloudy swelling in the myocardial and skeletal muscles.

IV. Diagnosis:

A. In the field.--In enzootic areas typical clinical features characteristic of the disease, such as dyspnea, edema of the supraorbital fossea, subcutaneous edema of the head and neck, respiratory and heart lesions, excess of pericardial and pleural fluids and severe gastritis, aid in forming a presumptive diagnosis.

B. Laboratory diagnosis.--Specimens required for laboratory examination in the study of AHS are:

1. Blood for virus isolation. Blood should be collected, with equal amounts of OGC fluid and kept refrigerated.

2. Tissues for virus isolation. Spleen and lymph nodes are suitable for virus isolation and may be collected in 50% neutral glycerin in buffered saline.

3. Serum for serological tests. Two or 3 serum samples are required; one to be collected at onset of disease; the second, one week after the decline of fever; and a third, 14 to 28 days following temperature peak.

Confirmation of the disease in the laboratory is arrived at by isolation of the AHS virus, using 3- to 6-day-old mice. The virus is then assayed in mice or cell culture and then identified by serological tests.

The most common and specific tests are the complement-fixation (CF) and virus neutralization (VN) tests.

Complement-fixing antibodies are of short duration and as the test is not AHS virus type-specific, it is invaluable in the diagnosis of the disease.

Virus-neutralizing antibodies appear shortly after the CF antibodies and persist for a prolonged period. The VN test also is invaluable for typing the AHS viruses.

C. Differential diagnosis*.--Although the epizootiological and clinical signs of African horse sickness may lead to suspicion of infection with the disease, clinical features and postmortem appearance of animals affected by the disease can be confused with other diseases to which equidae are susceptible, such as viral arteritis and equine infectious anemia.

1. Viral arteritis. Manifestations in animals which have been infected with viral arteritis are conjunctivitis, palpebral edema, and edema of the legs, abdomen, mammary glands, scrotum, and sheath. In this disease, the gross lesions may include petechial hemorrhages on the serous surfaces, and edema. Yellow, gelatinous infiltrations are observed in the mediastinal tissues, mesentery, and sublumbar tissues also.

Virological and histopathological examinations are required for differential diagnosis.

This disease is transmitted by contact, and it is not fatal to suckling mice.

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*See also appendices A and B.

2. Equine infectious anemia (EIA). The acute disease is characterized by sudden onset and a rise in temperature--usually to about 105 to 108° F. The affected animal may show continuous fever or frequent intermittent attacks. The characteristic epizootiology of EIA may lead to diagnosis of this disease. Infectious anemia is suspected when disease breaks out among several horses in a group brought together from widely scattered areas for racing, horse shows, country fairs, and rodeos. A few horses may die at the beginning of the outbreak, but it is usually followed by the subacute form and subsequent recovery. The particular nature of the petechial and ecchymotic types of hemorrhages on the surfaces of the major parenchymatous organs and serous and mucous membranes are distinguishing features.

V. Prognosis:

In susceptible equine populations the fatalities range between 80-90%. In enzootic areas, the mortality rate is modified in proportion to the resistance acquired by the equine population as a result of previous exposure or natural resistance.

VI. Epizootiology:

A. Geographical distribution.--African horse sickness was confined to certain regions in Africa until the summer of 1959 when it appeared in Iran, West Pakistan, and nearby Afghanistan. Instead of dying out over the winter as expected, it appeared in the spring of 1960 and rapidly spread to India, Turkey, Cyprus, Iraq, Syria, Lebanon, and Jordon. Thus, it appears that the disease can exist permanently in any area of the world where climatic conditions favor survival of culicoides, the principal insect vectors, throughout the winter months.

The role of climatic conditions seemed important in the occurrence of new outbreaks and the spread of the disease. It was observed in the 1966 disease epizootic in Tunesia, Algeria, Morocco, and Spain that many outbreaks of AHS arose after rainfall which was followed by 2 weeks of temperate, dry weather. This favored the rapid multiplication of insects.

B. Transmission.--Although various insects have been incriminated, culicoides are the chief vectors for the transmission of African horse sickness. However, there is insufficient information as to the chief reservoir of the virus. It appears that when the virus is maintained in an invertebrate, with optimal climatic conditions and a susceptible equine population, the disease makes its appearance. Equidae appear to be dead-end hosts although unvaccinated, susceptible animals could serve to amplify and enhance viral activity, especially in the presence of large numbers of easily infected culicoides.

C. Host range.--Equidea were found to be naturally susceptible, with horses and ponies the most susceptible. The Middle Eastern donkey has been found much less susceptible than the mule, but more susceptible than the African donkey which is quite resistant.

Dogs exhibit transitory viremia following ingestion of large quantities of meat and blood from infected equines.

VII. Control and eradication:

A. Preventive measures.--Although chronic carriers in AHS are not known, importation of equidae from countries in which the disease is enzootic to those free from it must be restricted. If importation is allowed the animals should be quarantined 30-60 days. Animals in quarantine must be maintained free from external parasites and protected from biting insects.

B. Natural immunity.--Equidae recovered from the disease develop solid immunity against the homologous strain of virus but may be susceptible to infection by other virus strains. Foals from immune dams have a natural immunity which protects them for the first few months of life and cannot be successfully vaccinated until their maternal immunity has waned at about the 8th month.

C. Induced immunity.--McIntosh reported 42 immunologically distinct strains of virus which could be divided into 9 virus types. The work of Alexander has made possible the production of an attenuated virus vaccine. A vaccine is produced by serial intracerebral propagation of the virus in mice. At the 100th serial passage, the virus is sufficiently attenuated to produce effective vaccine. The virus becomes neurotropic to mice and loses its virulence for the horse.

A polyvalent vaccine of 6 virus types has been used successfully to protect susceptible animals in South Africa.

More recently, the neurotropic mouse-adapted virus was propagated in cell culture and used in the 1966 disease outbreak in Tunesia, Algeria, Morocco, and Spain.

African horse sickness vaccine inactivated by formalin has been tried, giving protection to susceptible horses. The potential of such a preparation has not been fully evaluated in the field to determine its limitations and advantage.

There is always concern regarding a possible return of virulence during serial passage of the neurotropic virus in equines and many countries that are free from the disease would not accept a live vaccine as a prophylaxis.

The extensive spread of the disease in the Middle East and North Africa attests to the problems involved in its confinement, and confirms the necessity for constant vigilance to prevent the introduction of animals from infected areas into clean areas.

VIII. Public health aspects:

The disease is not transmissible to man.

Selected References:

1. Merchant, I. A. and Barner, R. D. 1964. African Horse Sickness. In "Infectious Diseases of Domestic Animals." Iowa State University Press, Ames, Iowa. pp. 417-421.

2. Maurer, F. D. and McCully, R. M. 1963. African Horse Sickness. Am. J. Vet. Res. 24:235-266.

APPENDIX A

DIFFERENTIAL DIAGNOSIS OF AFRICAN HORSE SICKNESS (AHS)

DISEASE FEATURE	DISEASES				
	AHS	EVA*	EIA**	TRYPS.	PIROP.
Clinical					
subc. edema	head, ventral body surface	ventral body surface tendon sheaths	abdomen & legs	generally distributed	
	++++	+++	+++	++	
Infra-orbital forces	++++				rare & late
PM lesions					
pulmonary edema	++++	++			rare & late
bronchopneumonia	+	+++			
hydrothorax	+++	++			
edema of pharynx	++++	++			
edema of intestines	+++	++			
edema general tissue	yellow & gelat. ++++	yellow & gelat. ++	watery +		
hemorrhages	++++		+++		
ictrus			++		
Epidemiology					
seasonal	+++		+++		+++
recurrent illness			+++	+++	+++
Microscopic exam					
arteries		+++			
bone marrow			+++		++
blood smears				+++	+++

*Equine Viral Arteritis

**Equine Infectious Anemia

APPENDIX B

SPECIMENS AND APPROACH FOR LABORATORY DIAGNOSIS OF EQUINE DISEASES WHICH MAY BE CONFUSED WITH AFRICAN HORSE SICKNESS

Diagnosis Suspected	Source of Material		Laboratory examination	
	Clinical	Postmortem	Antigen (virus)	Antibody (convalescent serum)
African horse sickness	Blood	Spleen	Mouse inoculation Equine transmission Cell culture	CF test, Mouse inoc. Tissue culture VN test, FA, AGDP
Equine infectious anemia	Blood and serum	Spleen	Equine transmission	AGDP test
Viral arteritis	Nasal swabs	Spleen	Cell culture Equine transmission Histopathology	VN test - tissue culture
Babesiosis	Blood smears	Spleen	Microscopic	
Anthrax	Blood and tissue	Smears, swabs, tissues	Microscopic culture Animal inoculation FA test	
Venezuelan equine encephalomyelitis	Blood, serums	Brain	Laboratory animals, cell cultures, Histopathology	VN test

AFRICAN SWINE FEVER *

I. Identification of disease:

A. Definition.--In its most usual and distinctive form African swine fever (ASP) is a highly contagious, peracute, febrile, and septicemic viral disease of domestic swine that is characterized by marked hemorrhages in the internal organs, cyanosis of the skin, and mortality closely approaching 100%. However, in areas where the disease has become enzootic in domestic swine, the mortality may be somewhat reduced and an increased frequency of subacute and chronic infections may be encountered. In any case, laboratory tests are usually required to establish positive differentiation between African swine fever and hog cholera (HC).

B. Etiology.--The virus of ASF (ASFV) is an icosahedron 175 to 215 nm in diameter and has an outer envelope acquired from the cytoplasmic membrane of the host cell. It is sensitive to lipid solvents and contains deoxyribonucleic acid (DNA). It is neither morphologically nor immunologically related to the virus of HC, nor is it related to any other mammalian virus presently known. It replicates entirely within the cytoplasm of the host cell. It is the only so-called icosahedral cytoplasmic DNA virus known to infect mammals. Other viruses of this type are found in amphibians, fish, insects, and plants.

In Africa, ASFV has been found in three species of indigenous wild pigs. They serve as reservoirs of the virus and apparently tolerate the infection without suffering ill effects. Yet, when this virus is transmitted to domestic swine, it usually gives rise to the lethal, peracute form of the disease.

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*Prepared by W.R. Hess, I.C. Pan and C. J. DeBoer

The Argasid ticks, Ornithodoros erraticus in Spain and Ornithodoros moubata porcinus in Africa, are capable of transmitting ASFV. The virus is known to persist for at least a year in the ticks and virus replication and transovarial infection have been demonstrated in them. They must, therefore, be regarded as reservoirs as well as vectors of the virus.

The serums of chronically infected pigs, or pigs that have survived infections with laboratory-modified strains of ASFV, usually contain antibodies capable of forming precipitates with a number of ASFV-associated antigens. Although virus neutralizing antibodies have not been convincingly demonstrated, these animals are often refractory to large challenge doses of the fully virulent homologous virus isolate. However, when challenged with a virus isolate from a different geographical location, the animal may die with the typical acute disease. It is therefore thought that several immunologic types of the virus may exist.

C. History.--The disease was first described by Montgomery who studied it extensively in Kenya during the period from 1910 to 1915 when outbreaks involving 1366 swine occurred with a mortality of 98.9%. He established its viral nature, determined it to be immunologically distinct from HC, studied the survival of the virus under a variety of environmental conditions, explored methods of transmission and immunization, studied its host range and suggested the possible role of wild swine in maintaining the disease in nature.

In the years from 1915 to 1957, outbreaks of ASF occurred in a number of areas south of the Sahara in Africa, and the recognition of the disease's potential threat to the world's swine industry began to emerge. During this period Montgomery's findings were confirmed. The wart hog and

bush pig were found to be inapparent carriers of the virus in Africa, but the mode of transmission from the wild to domestic pig remained obscure. Neutralizing antibodies were not found and though all available adjuvants and inactivation methods were tried, a suitable vaccine was not developed.

The USA was apparently the first major swine-raising country to fully recognize the destructive potential of ASF and assigned investigators to study the disease in Kenya in the early 1950's. However, it was not until the disease appeared in Portugal in 1957 and again in 1960 and then spread into Spain that it aroused the concern that it rightly deserved. Fortunately, workers in Kenya developed diagnostic procedures that greatly enhanced efforts to control and eradicate the disease, and for a few years it was successfully confined to the Iberian peninsula. But it had become established as an enzootic disease in Spain and Portugal, and perhaps through continued residence in domestic swine and the disasterous application of so-called modified live vaccines, the disease picture began to change. Subacute and chronic infections became more prevalent and its similarity to HC was even more pronounced.

In 1964, outbreaks of ASF were reported in France along the Pyrennes and in the Department of Brittany. Thanks to an early diagnosis and drastic slaughter program in which all infected and exposed animals were eliminated without further efforts to distinguish between ASF and HC, the disease was quickly eradicated. Outbreaks were reported in Italy in 1967 and 1969. Again, drastic slaughter programs were credited with eliminating the disease. The disease has also been reported in Madeira and in 1971 a major incursion of ASF occurred in Cuba. With technical assistance from Russia, France, and Canada, the disease was apparently eradicated in Cuba but only after nearly 400,000 pigs had died or were slaughtered.

At the present time, ASF still persists in Spain and Portugal, and very recently another outbreak was reported in France close to the Spanish border in the vicinity of Bayonne.

II. Signs:

A. Clinical features.--In peracute cases, death sometimes occurs without any apparent signs of disease other than fever and occasionally gross lesions that are hardly apparent. In acute cases, usually there is an abrupt and severe pyrexia which persists for 3 or 4 days. During this period the animals usually continue to eat and appear quite normal. Within 24 to 48 hours before death, temperature of infected swine begins to fall. They stop eating and lie huddled together. The pulse and respiration are accelerated. If forced to rise, they appear weak and poorly coordinated. Cyanotic areas are often present in the skin on the ears and other extremities. Other signs sometimes encountered are mucopurulent nasal and conjunctival discharges, vomiting, and diarrhea. Death usually occurs by the seventh day after the onset of fever.

In the subacute form of ASF, a high fever again marks the onset of the disease. However, the fever may persist for several days or fluctuate irregularly throughout the course of the disease which is usually 3 or 4 weeks. As with the acute form of the disease, there are no features that might clearly distinguish it from HC.

The manifestations of chronic ASF are extremely variable and the disease is usually difficult to recognize. The illness may persist for several months and stunting or emaciation may be the only apparent signs. Pneumonia is the most frequent sign, but occasionally arthritis and large cutaneous ulcerations have been reported. When it has been possible to

observe chronically infected animals throughout the course of the disease, it has been noted that they go through recurring cycles of pyrexia. It is sometimes possible to isolate virus from the blood during the periods of high temperature.

B. Incubation period.--The incubation period following natural or contact exposure is usually 5 to 9 days. In enzootic areas where the disease may be somewhat modified, incubation periods of 8 to 15 days are reported and subacute and chronic infections are more frequent.

III. Pathologic changes:

A. Post-mortem lesions.--In acute ASF, the lesions seen at necropsy are indicative of septicemia characterized by acute infectious splenomegaly and hemorrhages in various organs. There is usually an excess of pericardial, pleural, and peritoneal fluids. Although hemorrhages may occur in nearly every organ, petechiae are frequently observed on the renal cortex beneath the capsule, the mucosa of the urinary bladder, lungs, myocardial, subendocardial, and epicardial surfaces of the heart. The spleen is severely engorged and enlarged; infarction is not observed. Although acute erysipelas may also show infectious splenomegaly and generalized hemorrhages in the organs, petechiae on the renal cortex is more variable in size and irregular in shape. Marked ecchymotic lesions on the gastric mucosa are not seen in acute cases of ASF, while marked reddening of the gastric mucosa is common in acute erysipelas.

Nearly all regional and hilar lymph nodes of organs show swelling and peripheral reddening. The renal and hepatogastric nodes show the most severe hemorrhage and swelling and sometimes resemble blood clots. The mesenteric and pulmonary lymph nodes are less involved. The lungs are

usually edematous. The gall bladder is usually distended with bile and sometimes the wall is edematous. Petechiae are sometimes scattered over the mucosal surfaces and rarely the whole bladder is filled with the clotted mixture of blood and bile. "Paint brush" petechiation is usually present on the gastric and intestinal serosa.

Although the distribution and frequency of the lesions may vary considerably, depending on the strain of virus involved, certain of them occur more often than others and some of the gross lesions when present are considered pathognomonic. In this regard, acute infectious splenomegaly, marked swelling and hemorrhages of lymph nodes, and uniform-sized petechiae no larger than a pinhead on the renal cortex are considered to be most indicative of acute ASF.

In the subacute form of ASF, the incubation period is slightly increased. The disease starts with the onset of high fever. The total course is usually run within 3-4 weeks after infection. As in the acute disease, the reticuloendothelial system is involved and death is possibly due to vascular damage. Hemorrhages are more pronounced in lymph nodes and kidneys. In the latter, the cortex may resemble that of "turkey-egg kidney" and there may be extensive hemorrhage in the pelvices. Moderate edema in the perirenal connective tissue is usually present. Swollen spleen is usually due to hyperplasia of cell elements rather than engorgement. Lobular consolidation is usually observed in the anterior and cardiac lobes of the lungs. The entire lung may be white and does not collapse when the chest cavity is opened. This is due to the presence of diffuse interstitial pneumonia caused by ASF virus infection. Mucosal hemorrhages and bloody contents are often present in the large intestines.

As stated previously, stunting and emaciation are often the only apparent signs in pigs chronically infected with ASFV. Some of these animals may die following one of the frequent recurrences of pyrexia. In such cases, the gross lesions usually resemble those of the subacute disease. Hemorrhages may be prominent, but again any enlargement of the nodes or spleen is due to hyperplasia rather than engorgement.

If the chronically affected animal is killed for necropsy, hyperplasia of lympho-reticular tissue is the most prominent feature noted. The lymph nodes may be considerably enlarged and of very firm consistency. Chronic fibrinous pericarditis and pleuritis are often present. Frequently, firm foci varying in size from a single to several lobules are scattered in the lungs. Often, the lung does not collapse. The nodular lesions may coalesce to form a hard, white mass involving an entire lobe. There are no predilection sites for these foci. The lesions are produced by the infiltration of large numbers of mononuclear cells into the alveolar walls and lumens. Subsequently, the lesions become caseous masses in which calcification may occur.

B. Microlesions.--The virus of ASF acts almost exclusively on cells of the reticuloendothelial system. This is obvious in the histopathology of the acute disease. Severe degenerative changes occur in the lymphoid tissues, including spleen and lymph nodes. Perivascular cuffs of mononuclear cells with frequent karyorrhexis are seen in the brain, and fibrinous necrosis of vascular walls, accompanied by hyperemia and mural thrombiosis are frequently widespread in other organs.

Hyperplasia of lymphoid elements of the reticuloendothelial system are apparent in subacute and chronic ASF. In cases which have died following recurrent fever, degenerative lesions are superimposed on the hyperplastic

changes. The lobular consolidation often seen in the anterior and cardiac lobes of the lungs in the subacute disease show varying degrees of alveolar wall thickening due to mononuclear infiltration; edematous alveoli contain moderate numbers of degenerating mononuclear cells. In the chronic disease where the more advanced necrotic lesions of the lungs are present, the lesions are encapsulated and the adjacent lobules display perivascular and peribronchiolar hyperplasia consisting mostly of plasma cells.

Although the histopathology of ASF is of considerable interest to investigators studying the pathogenesis of the disease in its various forms, it is of little value in differential diagnosis and cannot be relied upon for that purpose.

IV. Diagnosis:

A. In the field.--There are a number of disease conditions in swine which may duplicate some of the signs of ASF, but the main diagnostic problem is in distinguishing it from HC. If epizootiologic events suggest the possibility of an ASF outbreak in an area, any febrile, hemorrhagic syndrome occurring in swine should be regarded as highly suspicious. This attitude should be reinforced by the fact that HC has been reduced to a rarity in this country while ASF has struck as close as Cuba.

Diagnosis of the acute form of the disease as it usually occurs in Africa is not especially difficult and a provisional diagnosis based on the history of the outbreak, the clinical signs, and port-mortem lesions is usually correct. However, the somewhat modified strains of the virus present in areas where ASF has become enzootic in domestic swine usually give rise to a disease that is impossible to differentiate from HC or a number of other swine diseases by clinical signs or pathologic changes. In any case, differentiation requires laboratory confirmation.

B. Laboratory.--A positive diagnosis of ASF requires either detection of the virus or demonstration of the presence of ASF-specific antibody. Several excellent tests are now available, and the evidence required for a positive diagnosis may be detected in any one of several kinds of samples. However, speed sensitivity and accuracy are considerations that govern the selection of samples to be submitted and the test to be conducted.

In acute ASF, the virus may be detected in or isolated from practically every organ, but spleen, liver, lymph nodes, and blood have the highest virus concentrations and are the tissues of choice for diagnosis. For detection of virus, immunofluorescence is the fastest method. Spleen and especially liver are the best tissues for this purpose. In situations where samples may be dispatched to the laboratory within a day or so, pieces of fresh tissue (3 or 4 grams) placed in separate vials and packed in ordinary ice are most satisfactory. About 10 ml of whole blood should also be sent in this manner.

If a longer time is required for transmitting the samples to the laboratory, they should be frozen and shipped on dry ice. In this event, pieces of liver and spleen about a centimeter square should be wrapped separately as cubes in aluminum foil before placing in vials and freezing. Other pieces may be frozen together in a vial. If freezing is necessary, a serum sample should also be sent.

Upon receipt in the laboratory, frozen sections of liver and spleen are cut and stained with fluorescent conjugate prepared from ASF-specific antiserum. Other pieces of tissue are ground together in whole blood to form a suspension that is inoculated on swine leukocyte cultures, and if available normal pigs and pigs immunized against HC are also inoculated.

Serum samples are tested against a preparation of ASF-associated soluble antigens by reverse radial immunodiffusion or immunoelectroosmophoresis to detect the presence of ASF-specific antibodies.

If subacute or chronic ASF are suspected, samples of affected lung tissue, if present, as well as spleen, liver, and lymph nodes should be sent to the laboratory in the same manner as indicated above. A serum sample, however, is absolutely essential, for the detection of antibodies is often the most rapid and certain means of diagnosing the slower forms of ASF.

C. Differential diagnosis.--As indicated previously, pigs dying of acute erysipelas may display post-mortem lesions similar to those of acute ASF, but the petechiae on the renal cortex are quite variable in size and shape while those of ASF are of a uniform size no larger than a pinhead. Also, in contrast to ASF, there is commonly a marked reddening of the gastric mucosa in acute erysipelas. In any case, the laboratory tests readily differentiate the two disease.

V. Prognosis:

Among pigs contracting ASF, mortality is essentially 100%. Although there are occasional survivors, they usually prove to be chronically infected and should be eliminated as potential spreaders of the disease. Slower or apparently milder forms of ASF occur, especially in areas where the disease has become enzootic in domestic swine. Here again, the survivors must be regarded as carriers that should be eliminated if the disease is to be eradicated.

VI. Epizootiology:

A. Geographical distribution.--Outbreaks of ASF have occurred throughout most of Africa south of the Sahara wherever domestic swine have been raised in close proximity to the indigenous wild swine. In parts of Angola and Mozambique the disease is enzootic in domestic swine, and in certain areas of Angola it is said that swine raising is virtually impossible because of ASF. The disease is also enzootic in parts of Spain and Portugal and outbreaks have been reported in Madeira, Italy, and France. The only occurrence of ASF thus far reported in the Western Hemisphere was in Cuba in 1971 and it has apparently been eradicated there.

B. Transmission.--It appears that arthropod vectors or the ingestion of infected tissues may be required for transmission of ASF from the wild to domestic swine in Africa. It is known that at least two species of Argasid ticks not only serve as vectors, but are reservoirs of the virus as well. Once it is established in domestic swine, the disease spreads readily among them by direct contact. The virus present in the excretions and secretions of infected animals is apparently transmitted to other animals by nuzzling or ingestion. The disease does not appear to spread readily by aerosol, for it has been shown experimentally that transmission may not occur between sick and normal pigs kept in separate cages in the same room. However, when the normal pig is allowed to come in contact with the excreta of sick animals, transmission usually occurs. Mechanical spread of the virus can obviously occur. The feeding of raw garbage containing infected meat scraps is an experimentally proven mode of transmission and has been strongly implicated in the spread of ASF from Africa to Portugal and from southern Spain to northern regions of the country.

C. Hosts.--Natural infections with ASFV appear to be confined to porcine species and to certain ticks. In Africa, the virus has been found in wart hogs (Phocochoerus sp.), bush pigs (Potamochoerus sp.), and giant forest hogs (Hylochoerus sp.). These species serve as a reservoir of the virus without displaying signs of illness. Argasid ticks, Ornithodoros moubata porcinus, collected from animal burrows in Africa have been found harboring the virus. In them, the virus replicates and transovarial infection occurs. A similar tick, Ornithodoros erraticus, in Spain has also become infected and has greatly complicated the task of ASF eradication in Spain and Portugal.

The European wild boar (Sus scrofa ferus) is susceptible to ASF and may acquire the infection through contact with infected domestic swine. The response to infection is like that of domestic swine and the lesions are similar.

The virus was apparently isolated in Africa from a hippopotamus, a porcupine, and a hyena. However, these findings have not been substaniated by additional isolations nor have efforts been made to experimentally infect these species.

Among a wide variety of mammals and fowl that have been subjected to virus inoculation, only rabbits and goats have been successfully infected. In both instances, the infections were difficult to produce. Natural infection has not been found in either species.

VII. Control and eradication:

A. Preventive measures.--The first line of defense, of course, should be set against entry of the disease. To this end, there must be a constant awareness and appraisal of the epizootiology of ASF, and importation restrictions must be placed on swine and pork products from

affected areas. All overseas garbage should be destroyed, preferably by incineration, and garbage feeding in general should be restricted to establishments that can assure thorough cooking. Any expertise that is possessed in diagnosing ASF should be freely shared.

It is hoped that the kind of surveillance and reporting of suspicious swine diseases that has been such an important part of the HC eradication program will continue with ASF in mind as well.

B. Sanitation and disinfection.--If an outbreak does occur, the "stamping out" method of eradication must be immediately applied. All sick and exposed animals must be eliminated and disposed of on the affected premises by burning or deep burial. The infected premises should be thoroughly cleaned and disinfected. Manure should be burned or saturated with disinfectant and buried. It has been found that disinfectant solutions containing o-phenylphenol and surface active agents combined are effective in destroying ASFV. In addition, the floors and walls of buildings that have housed swine should, if possible, be thoroughly scalded with live steam.

At least 6 months should elapse before restocking with swine, and during that time, several applications of a pesticide that is capable of destroying ticks is strongly recommended.

All premises surrounding the infected premise must be regarded as being in the affected area and must be kept under close surveillance. The movement of animals from the area must be restricted until it is declared free of the disease.

Every possible effort should be made to prevent the virus from becoming established in an arthropod population. Entomologists especially familiar with the tick species in the area should be consulted.

C. Treatment.--There is no known treatment for ASF.

D. Immunization.--All efforts to produce vaccines with inactivated virus have failed. Several isolates of ASFV have been modified by passage in cell cultures and rabbits and at least two of these have been used as vaccines with disasterous results. They not only failed to protect but quite probably were responsible for the increased incidence of chronic infections now encountered in the areas where they were used.

VIII. Public health aspects:

There have been no reports of ASFV infections in man despite the fact that large quantities of infected pork have been consumed in Africa, Portugal, and Spain and a considerable amount of laboratory work has been done with the virus.

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CONTAGIOUS BOVINE PLEUROPNEUMONIA*

I. Identification of the disease:

A. Definition.--Contagious bovine pleuropneumonia (CBPP), also known as lung sickness of cattle and longsiekte, is a highly infectious disease primarily affecting cattle. The disease may be acute, subacute, or chronic and is characterized by edema of the interlobular and alveolar tissues of the lungs, as well as the sero-fibrinous pleuritis.

B. Etiology.--The causative agent for CBPP is Mycoplasma mycoides var mycoides, also called by the earlier nomenclature, PPLO (pleuropneumonia-like organism). It is extremely pleomorphic and some of its forms are filterable. Mycoplasma are penicillin resistant and grow extracellularly in serum enriched media; colonies have a distinctive "fried-egg" appearance on agar plates.

The disease occurs commonly only in cattle with rare natural cases being observed in buffaloes, yak, bison, reindeer, and antelopes. The causative organism of contagious pleuropneumonias in cattle and goats are similar, but the infections do not spread between the two species.

C. History.--The first records of the existence of CBPP were in Germany in 1693 and in 1743. From here the disease spread in epizootic form over the whole of Europe. The disease was first introduced into the USA in 1843 and eventually resulted in the establishment of the Bureau of Animal Industry in 1884. The disease was completely eradicated from the USA by 1892, from South Africa in 1916, and Australia in 1971. The disease is now primarily confined to Africa and Asia; in Europe it is confined to Spain and Portugal.

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*Prepared by R.J. Yedloutschnig and F.W. Wilder

II. Signs:

A. Clinical features.--In the acute form there is a sudden onset of high temperature (105 F), anorexia, severe depression, and a tendency for animals to stand away from others. Chest pain is evident with the affected animals reluctant to move. Their characteristic stance is with elbows out, back arched, and head extended into the wind. Respirations are shallow and rapid; coughing occurs only when the animal is forced to exercise.

Recovered animals may be clinically normal but they remain carriers. Sequestrae in these carriers may break down upon stress or severe exercise and cause an acute outbreak of the disease. A chronic cough is common. Approximately 50% of affected animals die in the acute stage and about 25% remain as recovered carriers. Death occurs over a course of from several days to 3 weeks.

B. Incubation period.--Under natural conditions the incubation period of the acute form is 3-6 weeks, with an occasional period up to 6 months. The chronic disease may persist for 2 years or longer.

III. Pathologic changes:

A. Post mortem lesions.--Lesions are confined to the thoracic cavity. Pleuritis with heavy deposits of fibrin are seen. One or both lungs may be partially or completely affected with marked consolidation. Interlobular septa are greatly distended with serofibrinous exudate--the classical "marbled" lung. Sequestrae of carriers may be deeply embedded in the lung and no abnormality seen on the surface. Adhesions between pleural surfaces are often seen in such cases.

B. Microlesions.--Lung lobules are frequently separated into distinct compartments by heavily thickened interlobular septa. Some lobules

contain areas of intact alveoli, but in many, consolidation is complete. Intense infiltration with lymphocytes and plasma cells may be noted around blood vessels and bronchi. Leukocytes may be concentrated within the interlobular septa.

IV. Diagnosis:

A. In the field.--Clinical signs are indistinguishable from other diseases which produce a severe pneumonia. Observations of the more or less typical marbled appearance of affected lobules and the excretion of excessive amounts of straw-colored thoracic fluid should lead to suspicion of CBPP.

B. Laboratory.--Serological tests such as complement fixation, precipitin, and agglutination tests are used for laboratory detection of antibodies. Fluorescent antibody techniques give excellent results for detecting the organism in tissues as well as classification by means of colonies on agar.

C. Differential diagnosis.--Any disease producing respiratory problems may be mistaken for CBPP. This includes shipping fever, Pasteurella sp., pneumonia, and many other disease entities.

V. Prognosis:

Death usually occurs in 2 to 3 weeks after the initial signs are observed in the acute disease; mortality varies from 10% to 70%. Animals which do not die may make an apparent recovery; this is frequently nearly half of the affected animals.

VI. Epizootiology:

A. Geographical distribution.--The disease occurs mainly in most parts of Africa and Asia (including Indochina and asiatic USSR). In Africa, the Sudan and Ethiopia have major foci. Cattle in Spain are also affected.

B. Transmission.--The disease is spread through inhalation of aerosols into the respiratory tract. Foci of new outbreaks may result from carrier animals when infective particles are released by respiratory expulsion of material from sequestrae which have broken down.

C. Hosts.--Cattle of all ages are susceptible; most other ruminants are considered resistant. Sheep and goats never contract the natural infection.

VII. Control and eradication:

A. Preventive measures.--Preventing overcrowding and quarantining of apparently sick animals or others which might serve as contact carriers, especially, are important measures. It is especially important to keep recovered animals away from susceptibles because of the extended carrier state.

B. Sanitation and disinfection.--The Mycoplasma are especially susceptible to sunlight and are quickly destroyed in nature. They are not, apparently, spread throughout the carcass. Transmission is largely by aerosol.

C. Treatment.--Sulfonamides and broad spectrum antibiotics have been used in enzootic areas as a means of salvaging meat. However, this should be discouraged because of the production of carrier animals. Slaughter is most economical in small foci of infection.

D. Immunization.--The best vaccines are prepared from live, modified cultures of M. mycoides var mycoides. Several strains are utilized depending on the breed of cattle and the stability of the vaccine. In enzootic areas of CBPP, vaccination will continue to be important.

VIII. Public health aspects:

There is no evidence that man is susceptible.

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DERMOPATHIC BOVINE HERPES VIRUS INFECTION *

I. Identification of disease:

A. Definition.--Dermopathic bovine herpes virus infection (also known as Allerton virus disease, bovine herpes mamillitis, pseudo-lumpy skin disease) is a disease of cattle caused by a herpes virus which may produce a mild to severe mamillitis. It is characterized by a generalized condition of bumps on all parts of the skin not accompanied by other appearance of illness except a mild transient rise in body temperature.

B. Etiology.--The etiologic agent has been identified as a virus belonging to the herpesvirus group.

C. History.--One of the cytopathogenic agents recovered from lumpy skin disease of cattle from Africa was classified as a Group II (Allerton) virus. Bovine mamillitis has been reported in Britain since 1958, but confirmation of a herpesvirus as a causative agent was first announced in 1963. The disease also occurred in Scotland at that time, again in 1964, and with increasing frequency in 1965. During the next five years its frequency decreased. This herpesvirus was next isolated from cattle in the USA in 1970 and from animals in Italy in 1972.

II. Signs:

A. Clinical features.--In Britain and Scotland natural infections have been associated only with mamillitis in dairy cattle. The Allerton virus and isolates from the USA produce a generalized "lumpy skin disease" as well.

B. Incubation period.--In natural infections the incubation period is 5-10 days.

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* Prepared by R.J. Yedloutschnig

III. Pathologic changes:

A. Post mortem lesions.--The major lesions are eruptions of the skin and, where there is mamillitis, inflammation of the dermis of the teats and udder. This may vary or progress from a mild inflammation to an ulcerative condition.

B. Microlesions.--Syncytia formation and necrosis of the epidermis may be found. Many cells and syncytia have central nuclear inclusion bodies.

IV. Diagnosis:

A. In the field.--Demonstration of small nodular eruptions all over the body of bovidae, or inflammation of teats and udder where mamillitis is involved.

B. Laboratory.--Attempts should be made to isolate the herpesvirus and carry out neutralization of the virus in tissue culture. The demonstration of herpesvirus by electron microscopy is helpful. Reproduction of the disease in bovidae confirms the diagnosis.

C. Differential diagnosis.--This disease may be confused with that caused by Dermatophilus congolensis or other fungal or bacterial conditions; urticaria, cowpox, or the true lumpy skin disease (Neethling virus) also cause similar lesions.

V. Prognosis:

Mamillitis cases may be severe, with mastitis; one quarter may even be lost. The lumpy skin condition usually heals with no after effects.

VI. Epizootiology:

A. Geographic distribution.--Allerton virus is present in Africa; another virus causing mamillitis exists in Britain and Scotland. The herpesviruses recently reported from the USA and Italy are all similar.

B. Transmission.--The disease may be transmitted by direct contact and through milking. Biting insects are also considered to be a factor in transmission. Most outbreaks in Britain and Scotland have occurred between June and December.

C. Hosts.--The only natural hosts are apparently the bovidae. Mice and guinea pigs may be infected experimentally.

VII. Control and eradication:

A. Preventive measures.--Quarantine mamillitis cases; these should be milked last and away from the rest of the herd. The population of biting insects should be eliminated or lowered.

B. Sanitation and disinfection.--Equipment and hands should be thoroughly disinfected following use on infected animals.

C. Treatment.--Supportive therapy is recommended for the mamillitis or ensuing mastitis.

E. Immunization.--None.

VIII. Public health aspects:

Authorities are uncertain regarding the possibilities of human infection.

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FOOT-AND-MOUTH DISEASE *

I. Identification of Disease:

A. Definition.--Foot-and-mouth disease (FMD) is a contagious, viral infection primarily of cattle, swine, sheep, and goats, but also of other cloven-footed domestic and wild animals. It is characterized by vesicular lesions and subsequently by erosions of the epithelium of the mouth, nares, muzzle, feet, teats, udder, and rumen pillars.

B. Etiology.--Foot-and-mouth disease virus (FMDV) is classified along with the enteroviruses and rhinoviruses as a member of the picornavirus group. The virus is essentially spherical and is about 23 mμ in diameter.

C. Other vesicular diseases.--The other vesicular disease viruses have the following shapes and approximate diameters: vesicular stomatitis (VS) bullet-shaped, 65 x 175 nm; vesicular exanthema of swine (VES) spherical, 35-40 nm; swine vesicular disease (SVD) spherical, 28-32 nm. All of these viruses contain RNA. FMDV differs from most of the other picornaviruses (except rhinoviruses) by being more labile to pH changes. The organic acids (e.g., acetic acid) and strong bases (e.g., sodium hydroxide) are commonly used as disinfectants for FMDV.

At least 7 immunologically distinct types of FMDV have evolved, and they are identified as types O, A, C, SAT** -1, SAT-2, SAT-3, and Asia-1. Within the 7 types, at least 61 subtypes have been designated by complement fixation (CF) tests conducted by the FMDV World Reference Laboratory at the Pirbright Research Institute, Surrey, England and the Pan American FMD Center, Rio de Janeiro, Brazil.

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There are two immunological types of VSV, New Jersey and Indiana. Three subtypes of Indiana type virus have been identified, only one of which exists in the U.S.

VES virus is more mutagenic for at least 11 different immunologic types of the virus were identified before the disease was eradicated from U.S. swine in 1956.

The swine vesicular disease viruses are currently known only as viral isolates, from Italy, Hong Kong, England, France, Austria, Poland, Belgium, and Japan.

D. History.--The first acknowledged description of FMD was made by the monk physician, Hieronymus Fracastorius, who reported on an epizootic in cattle that occurred near Verona, Italy in 1514. For the next two centuries there was an ever increasing number of epizootics in cattle reported in various parts of Europe, many of which probably were FMD. The disease has long been present in Africa and Asia and most of South America. The United States has had 9 FMD outbreaks between 1870 (Oriskany, N.Y.) and 1929 (Los Angeles, Cal.). The 1914-1916 outbreak was the largest and most costly; FMD invaded 22 states and the District of Columbia, involving about 172,220 animals. The disease last occurred in Canada in 1952 and in Mexico from 1946 to 1954. The Central American countries from Guatemala through Panama have never had FMD, nor has New Zealand. Japan and Australia have been free of FMD for more than 50 years.

II. Signs:

A. Clinical features.--In the respective hosts of FMD, VS, VES, and SVD, the clinical signs and lesions so closely resemble each other that a differential field diagnosis is almost impossible to make. The classical signs of these vesicular diseases are salivation and lameness caused

by the formation of vesicles or blisters in the mouth and on the feet. However, before vesicles form there are usually some obvious signs of illness such as dullness, inappetence, uneasiness, a fall in milk yield in dairy cattle, fever, and sometimes shivering. A few hours later, quivering and smacking of lips, drooling, slight nasal discharge, shaking or kicking out with feet, or lameness may be noticed. After the vesicles have formed, the various signs usually are more pronounced, with salivation and often nasal discharge more copious and lameness more evident. Pregnant cows may abort and young animals may die without showing outward signs of infection. The mortality in mature animals seldom exceeds 5 percent. In young animals, the mortality may be as high as 50 percent.

The diagnostic lesions are epithelial vesicles or blisters. Lesions may be found on the tongue, dental pad, gums, cheeks, hard and soft palate, lips, nostrils, muzzle, snout of pigs, coronary band, corium of dewclaws, interdigital space, teats, udder, rumen pillars, and in the myocardium and skeletal muscles. With the exception of rumen, myocardial and other muscle lesions, VS, VES, and SVD produce lesions in the same anatomical areas as does FMD.

Often FMD lesions are found on all feet, but sometimes only one or two feet are involved. Swine often have lesions on the snout and tongue, but the diagnosis is usually based on the foot lesions. The most common site for oral lesions in sheep is the dental pad. In general, the signs and lesions seen in swine, sheep, and goats are similar to those found in cattle, but they may be less obvious.

B. Incubation period.--When susceptible animals are in contact with infected animals that are in the clinical stage of the disease, transmission

of FMDV readily occurs and recognizable clinical signs of FMD usually may be seen in the exposed animals within 3 to 5 days. Much longer incubation periods also have been reported. The peak period of transmission usually is about the time the vesicles rupture. Pigs fed garbage contaminated with FMDV may show signs of infection in 1 to 3 days. Artifically exposed animals may develop signs as early as 12 hours after inoculation, however, the usual interval is 24 to 48 hours.

III. Pathologic changes:

A. Pathogenesis.--Various studies have shown that the usual primary site of FMD infection and initial virus replication is in the cells of the mucous membrane of the throat.

The usual pathogenesis of FMD in cattle may be summarized as follows: 1) inhalation or ingestion of virus; 2) infection of cells in throat area; 3) replication of virus in throat area and spread to adjacent cells; 4) escape of virus to blood and lymph vessels; 5) infection of lymph nodes and other glands; 6) infection of cells at the sites of predilection for lesion development; 7) virus present in various body fluids; 8) beginning of fever; 9) appearance of oral, nasal, podal, and rumen vesicles; 10) appearance of salivation, nasal discharge, lameness; 11) rupture of vesicles and increased clinical signs; 12) end of fever; 13) end of viremia and start of detectable antibody production; 14) decline of virus titer in various tissues and fluids; 15) healing of lesions and resumption of eating; 16) gradual disappearance of virus in tissues and fluids; 17) healing completed but continued residence of virus in throat area with slow replication resulting in carrier state.

Secondary lesions of FMD are those that appear after there has been an initial lesion elsewhere. For example, if an animal is inoculated with FMDV in the tongue epithelium and this becomes the primary lesion, the other

later appearing oral, nasal, or podal vesicles are called secondary lesions. Teat and udder lesions may be primary as the result of virus contact during milking or by nursing infected young.

B. Postmortem lesions.--Rumen, myocardial, and skeletal muscle lesions occur in FMD infection, but not in VS, VES, or SVD. The rumen lesions (found on the pillars) begin as true vesicles similar to oral lesions, but have a thinner epithelial covering. The FMD myocardial lesions are areas of degeneration and necrosis, not vesicles.

Sometimes, especially in young animals, the so-called "tiger heart", with lesions in the form of stripes or bands, is found. Young animals that have died of FMD infection may have this extreme form of heart involvement.

C. Aftermath of FMD.--As an aftermath of FMD infection, animals may develop chronic secondary infection of their oral, nasal, or podal lesions. Hoof deformation may result in permanent lameness. Also, mammary gland involvement may result in chronic mastitis or a low milk yield. Unthriftiness and failure to regain weight are often seen. Sometimes this is associated with heart damage in FMD infections. Abnormal estrus or breeding problems may last for many months. The panting syndrome has been associated with pituitary gland involvement from FMD infection, resulting in an upset in the body heat regulatory mechanism. Diabetes mellitis also has been found as an aftermath of FMD.

IV. Diagnosis

A. In the field.--The typical vesicles, with blanched epithelial covering and filled with clear, colorless, or straw-colored fluid, are pathognomic of FMD, VS, VES, or SVD, and evidence of their existence is essential in clinical diagnosis of any of these diseases. After rupture of the vesicles, the lesions usually progress through necrotic, ulcerative and fibrinous

exudative stages. Occasionally, there are dry lesions that do not vesiculate. When the lesion is in these other stages or forms, diagnosis becomes difficult because other diseases may produce similar appearing lesions. The clinical signs of vesicular diseases, fever, salivation, nasal discharge, or lameness, are also produced by certain other diseases.

In cattle, oral and nasal lesions of rinderpest, infectious bovine rhinotracheitis, malignant catarrhal fever, viral diarrhea/mucosal disease, bovine herpesvirus infection/bovine mammilitis, lumpy skin disease, bovine papular stomatitis, infectious bovine ulcerative stomatitis, bluetongue, mycotic stomatitis, localized bacterial infections, and photosensitization could be confused with the later stages of FMD or VS. In addition to some of these diseases, foot rot, chorioptic mange, bovine pox, contagious ecthyma, ergot poisoning, and injuries from trauma or chemicals produce foot lesions that may resemble those of FMD or VS. In sheep, the other diseases that may cause diagnostic problems regarding FMD or VS are: sheep pox, bluetongue, contagious ecthyma, lip and leg ulceration and infection with Fusiformis sp. When only swine are involved, FMD, VS, VES, or SVD are clinically indistinguishable from each other and old lesions possibly could be confused with those of swine pox or injuries from trauma or chemicals.

B. Field samples.--Oral, nasal, or podal lesions may be used, but they should be fresh and representative. The following samples may be taken from each of 2 or 3 animals.

1. Vesicular fluid (quantity: all that is obtainable).

2. Vesicular lesion epithelial coverings.

3. Flaps of epithelial tissue still attached to the edges of the lesion (2nd choice)(quantity: from Items 2 and 3: 5.0 gm).* Old necrotic fibrinous material that is difficult to remove is undesirable.
4. Blood with anticoagulant added (quantity: 5.0 ml). Viremia ends about 4 or 5 days after onset of disease.
5. Oesophageal-pharyngeal (OP) fluid obtained with a cup-probang from cattle, sheep, or goats, but not from pigs (quantity: about 10.0 ml, before dilution).
6. Blood for serum samples (quantity: about 10.0 ml of serum).
7. From dead animals, a sample of lymph node, thyroid, adrenal, kidney, or heart may be taken (quantity: about 10.0 gm).

Samples of lesion epithelium, OP fluid, and serum always should be taken. In addition, if there are animals convalescent from the infection, serum samples should be taken from them. With the exception of serum samples, it is important that all other samples are promptly frozen and arrive at the laboratory in that condition.

C. Laboratory.--The field samples, i.e., vesicular fluid or lesion tissues, are prepared as the antigen for complement fixation (CF) tests with guinea pig reference serums for the various vesicular diseases. If the sample contains sufficient virus and it happens to be one of the 4 vesicular diseases, a differential interpretation can be made. In the case of FMD or VS, the virus type will also be known. Additional tests are required for VES and SVD and to determine FMD or VS subtypes. Some of the lesion tissues, OP fluids, blood with anticoagulant and organ tissues are

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*When lesion material is placed in a vial, 5.0 gm occupies a space about equal to 5.0 ml of water.

used for virus isolation. All viral isolates are subjected to CF tests for identification to confirm initial CF test results. Serum samples from convalescent animals may be used to differentiate the vesicular diseases in neutralization, agar gel diffusion or fluorescent antibody tests. These tests also may be used to identify viral isolates.

D. Differential diagnosis.--In the past, differentiation of the vesicular diseases was sometimes possible by animal inoculation, but modern rapid laboratory diagnostic tests has made this unnecessary in the field.

Differential Diagnosis of Vesicular Diseases

Based on Species of Animals Infected.

Diseases	Cattle	Sheep	Swine	Horses	Man
Foot-and-mouth disease	S	S	S	R	S***
Vesicular stomatitis	S*	S	S	S	S**
Vesicular exanthema of swine	R	R	S	E	R
Swine vesicular disease	R	R	S	R	E****

*IM inoculation of VS virus in cattle gives negative results.

S = susceptible, R = resistant, E = experimental transmission only.

**Vesicles rarely occur in man.

***Only a very few cases recorded in world's literature.

****Thus far, laboratory infections only.

V. Prognosis:

Prognosis for recovery in FMDV, VS, VES, or SVD is generally favorable except in very young animals or unusually severe epizootics. From the standpoint of eradication, however, prognosis is an insignificant factor since the first principle of success involves early discovery and prompt disposal of infected and exposed animals.

VI. Epizootiology:

A. Geographical distribution.--Currently (1972), FMD is found, and generally considered enzootic, in Asia, Africa, and most of Europe and South America. North and Central America, Australia, and many of the smaller islands of Oceania are free of FMD. The major livestock producing countries that are also free of FMD include: New Zealand, Japan, the Philippines, Norway, Ireland, and periodically Great Britain. Vesicular stomatitis is confined to the tropical and temperate areas of North, Central, and South America. In recent years, very few outbreaks of VS have occurred in the United States. It is now believed that VES may be an extinct disease in swine that was known to have flourished for only 24 years. The first outbreak of VES was discovered in California in 1932 and the last identified case involving swine occurred in New Jersey in 1956. Swine vesicular disease has been identified in Italy, Hong Kong, England, France, Austria, and Poland.

Interestingly, during the last year workers on the West Coast have isolated several viruses from two species of marine mammals that produce vesicles when inoculated into swine which are indistinguishable from those produced by FMDV, VESV, or SVDV. The chemical and physical properties of the viruses from marine mammals are also indistinguishable from those of VESV. Madin has theorized that marine mammals may be the reservoir for

VESV and that the disease may have originated in swine in California in 1932 as a result of feeding harbor seals to swine.

B. Transmission.--The primary method of transmission of FMDV from infected to susceptible animals is via respiratory aerosols. Aerosol transmission usually occurs with animals in close proximity. However, there is circumstantial evidence that animals may be infected from several yards to many miles downwind from a source of infection. In the British outbreak of FMD in 1967-1968, there is some evidence that contaminated milk aerosols from milk truck vents may have spread the disease to several farms. It has been experimentally demonstrated that when man inhales the respiratory aerosols of FMD-infected animals he may pick up and harbor the virus in his throat area for at least 24 hours. During this time, man may transmit the virus to other people and to animals via his respiratory aerosols. The OP fluids and respiratory aerosols from FMD-infected animals may contain virus before, during, and after appearance of clinical signs and lesions of the disease. Thus, normal appearing animals that have recovered from infection or were vaccinated for FMD and then exposed to the virus may harbor the virus in their throat areas for variable periods of time (about 6 to as long as 24 months in cattle, about 4 to 6 months in sheep and goats, but only during the clinical stage of the disease in pigs). There is circumstantial evidence that such carrier animals, especially cattle, may transmit the disease when introduced into a FMD free herd. However, experimental attempts to demonstrate transmission of FMD from carrier to susceptible animals have been unsuccessful. Experimental transmission of FMD from infected to susceptible animals has been successful only to about 8 days after the onset of the disease in the donor.

In addition to inhalation of virus, animals also may get their initial throat infection of FMDV by ingestion of contaminated forage, grain, animal products, or water or by licking contaminated objects (e.g., a German outbreak was traced to a bicycle). The virus also may gain entrance and establish the initial infection through abrasions in the mucous membranes or skin. It has been demonstrated experimentally that FMD may be transmitted by artificial insemination of infected semen. Meat scraps and bones from infected animals often have been the source of FMD infections in pigs, which then can readily transmit the disease to cattle and other animals. Several United States FMD outbreaks were traced to pigs that had been fed uncooked garbage from foreign ships. Outbreaks or spread of FMD have also been traced to the use of contaminated biological products (usually of foreign origin), such as: vaccinia vaccine, hog cholera vaccine, and pituitary extract. Since FMDV is present in much of the apparently normal skin, the salting, drying, or surface disinfection of hides from infected animals does not preclude virus survival. For short periods, virus may survive in wool. Imported endocrine glands and improperly dried blood are also potential sources of FMDV. Milk from infected animals sometimes contains a considerable amount of FMDV.

Outside the animal body, variable conditions affect virus viability. When exposed to sunlight, especially in a thin layer, the virus is readily destroyed, but in tissue fragments containing the virus or on material, such as hair, feet, and stable equipment, the virus may remain infective for several weeks under the usual stable and farm conditions. In one instance, on a farm in California, the virus persisted for 345 days.

C. Hosts.--Cattle, swine, sheep, goats, water buffalo, bison, deer antelopes, wild swine, reindeer, moose, llama, chamois, alpaca, vicuna, giraffes, and camels are generally considered the natural domestic and wild hosts of FMDV. Experimentally, FMD may be transmitted to mice, rats, guinea

pigs, rabbits, hamsters, embryonating chicken eggs, chickens, and various wild species, including European hedgehogs, chinchillas, muskrats, grissly bears, elephants, armadillos, and peccaries. Horses are resistant. Young dogs and cats may be infected by inoculation of virus, but probably do not contract the infection by natural means. When inoculated, FMDV will replicate without producing clinical signs or lesions of disease in monkeys, turtles, frogs, and snakes. Suckling mice have replaced guinea pigs for many FMDV studies. Guinea pigs are mainly used for production of diagnostic serums and in vaccine potency studies.

Generally, all susceptible animals in an exposed herd develop infection in time, but, under some circumstances, the incidence of disease is considerably less than 100 percent. Young animals are usually more susceptible than adults, unless protected by maternal antibodies arising from previous infection or vaccination.

To reduce viral aerosols in a FMD outbreak, the order of kill of infected and exposed animals should be: pigs, cattle, and sheep. Airborne FMDV may persist in animal rooms for at least 48 hours.

VII. Control and eradication:

A. Preventive measures.--The exclusion of animals of susceptible species and fresh meats therefrom, and restrictions on animal products originating in infected countries, constitute the most effective preventive measures. Included among these measures is the prohibition of garbage containing meats from infected countries aboard ships and aircraft arriving in the United States. Some products considered to be potentially dangerous, such as hides, bones, animal casings, and glands, are permitted entry into the United States but only under prescribed conditions wherein the products are processed at designated official establishments under supervision or comply with other regulations permitting their safe entry.

B. Control and eradication procedures.--

Reporting. If a vesicular disease or any foreign disease of animals is suspected, the owner should be requested to refrain from moving animals or animal products from the premises until the diagnosis is made and the veterinarian should promptly notify the nearest State or Federal veterinarian in his state. A specially trained diagnostician is available and will be sent to assist in the field diagnosis and collection of specimens for submission to the appropriate laboratory.

Special actions applicable to epizootics. In addition to prompt imposition of effective quarantines, immediate establishment of inspection procedures for the purpose of checking all possible contacts and cleaning and disinfection of the affected premises constitute the chief means of combating the disease.

Stamping-out method. Briefly, this technique consists of the following actions:

(1) Promptly slaughtering and disposing of animals infected with or exposed to FMD, thus removing at once the greatest source of active virus and avoiding the possibility of carriers.

(2) Carrying out thorough cleaning and disinfection of the premises and of material possibly contaminated with virus.

(3) Instituting prompt and meticulous tracing of contacts with infected herds and initiating a system of repeated inspections and surveillance in the areas involved.

(4) Imposing rigid judiciously designed quarantines for control of movement of people, livestock, animal products, and feeds.

(5) Indemnifying owners for animals, products, and materials destroyed in the course of eradication procedures.

(6) Testing the contaminated premises 30 days after disinfection by placing test animals, including cattle and hogs, to feed and graze and otherwise come in contact with all parts of the premises and objects which might have been contaminated with FMDV.

Since the 1902 outbreak, the stamping-out or slaughter method of eradication has been the established procedure in the United States where every practical means is used to keep the disease out of the country. If the infection were to enter again despite these protective measures, the same established procedures for eradicating the disease would undoubtedly be applied. General experience has demonstrated the practicality, economy, relative speed, and efficacy of these procedures. The infection has been eliminated through similar efforts in Canada. The British Government has reaffirmed a similar policy, even though the British Isles are almost continuously exposed. It must be realized, however, that elimination of a plague like FMD might present many new and complex problems today. For example, more livestock is being shipped greater distances in more ways and with greater speed than ever before. The control of animal transport is one of the most important functions of the livestock sanitarian. Another of today's problems is the burial and burning of large numbers of animals. Consideration must be given to the effect of such procedures on water and air pollution.

C. Treatment.--There is no known specific cure for the disease and the palliative treatment only alleviates the signs and does not prevent the spread of infection.

D. Immunization.--The use of vaccines in the control of FMD in Europe began about 1938. In Europe and South America, generally trivalent (i.e., with types A, O, and C) vaccines are used. In order to be

effective, vaccines must contain virus of the same type, and frequently of the same subtype, that prevails in the field. Like certain other viruses, FMDV is frequently being changed by mutations, natural passage through various species of animals, and probably by passage through carriers with varying levels of antibody protection. This necessitates frequent checking of at least primary outbreaks and widely scattered outbreaks; often the viral composition of the vaccines must be changed several times during the course of an epizootic.

Vaccinations should be controlled officially in a systematic manner. Under the best conditions, the vaccines for FMD are not infallible. Resistance induced by a good product wanes rapidly after 4 to 6 months; therefore, vaccination must be repeated at intervals. Even with modern laboratory techniques for producing virus for the vaccine, the cost of immunization is substantial. For example, in France, all cattle 5 months or older are vaccinated annually and the total cost for vaccinating one animal is approximately $1.00. In Argentina, the cost for one vaccination is much less; however, many estancias repeat the vaccination 3 times a year.

With ample supplies of safe potent vaccine, substantial control of FMD has been accomplished in several countries, and some have used vaccine as a supplementary, temporary adjunct to eventual eradication. If eradication is the goal, however, vaccination must be terminated eventually in order that the presence of the virus may be revealed by a totally susceptible population. Moreover, strong protective measures must be imposed or the disease may be reintroduced readily from infected areas; inevitably, eradication will be accomplished only through concerted regional or continental efforts.

E. Import Restrictions.--Restrictions on imports of animals and animal products are formulated and administered by the Animal and Plant Health Inspection Service of the U. S. Department of Agriculture, under the authority of Section 306A of the Tariff Act of 1930, and Acts of 1890 and 1903. The regulations are subject to revision from time to time in accordance with the requirements of changing conditions.

VIII. Public health aspects:

While man may harbor FMDV in his throat area for short periods of time, the temporary infection rarely becomes a clinical entity. Thus, the disease is not a public health problem.

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FOWL PLAGUE*

I. Identification of disease:

A. Definition.--Fowl plague (FP), also termed klassische geflugel-pest and peste aviaire, is a highly contagious, acute, fatal disease of chickens, turkeys, pheasants, and waterfowl. Guinea fowls, mynah birds, blackbirds, and sparrows are susceptible.

B. Etiology.--Fowl plague was first reported in Italy in 1878; in 1900, its viral etiology was proved. In 1955, it was shown that FP virus shared a common antigen with influenza A viruses and therefore it was classified as a member of that group of myxoviruses. Fowl plague virus is 80-120 mu in diameter having a single stranded RNA in its core, which is surrounded by a protein coat and an envelope. The viruses of fowl plague, Turkey/England/'63, Chicken/Scotland/'59, Tern/S. Africa/'61 and N virus all are members of the avian influenza A group of viruses. Infections with these viruses (except N virus, which produce 20% mortality in chickens) result in high mortality, up to 100% under natural conditions. However, the relationship among these strains are determined by analysis of the viral envelope antigens. Infectivity is rapidly destroyed by formaldehyde, detergents, halogens, and dilute acids. Fowl plague virus was viable after 15 minutes at 55° C, but was destroyed after 5 minutes at 60° C. At pH 4.0 it is destroyed within 60 minutes. The virus may survive on feathers for at least 18 days and in dried blood and tissues for several weeks. Its infectivity was retained in chilled meat and bone marrow as long as 287 and 303 days respectively.

C. History.--Disease epizootics in chickens, attributed to fowl plague, appeared in Italy just before the beginning of the 19th century

*Prepared by A.H. Dardiri

and shortly it was clinically differentiated from fowl cholera. Since then, it has been detected in Austria, Switzerland, Hungary, Rumania, and Russia. Occasionally, it has spread to France and Holland; it also has been reported in China, India, Indonesia, and Japan. The disease has been diagnosed in Egypt, Israel, and Iran and it may have also occurred in Nigeria. Outbreaks were reported in Argentina in 1922 and in 1924. The disease also appeared in England for the first time in 1924. Two outbreaks were reported in England in 1963 and they were promptly eradicated.

Following illegal introduction of fowl plague virus by a laboratory worker to the United States in 1923, the disease escaped from the laboratory and appeared in the New York City poultry markets in 1924, where it killed about one-half million birds. From there the disease spread to Pennsylvania, New Jersey, Illinois, Michigan, Missouri, West Virginia, and Delaware. The Indiana agent was probably transmitted by contaminated crates, live poultry shipments, and offal from canning plants. The disease was eradicated and controlled by slaughter, decontamination procedures, and rigid quarantine measures. The last outbreak in the United States was reported in Morris County, New Jersey, in 1929, and was eradicated before it spread beyond the limits of the county.

II. Signs:

A. Incubation period.--It is usually 3-7 days, but may be as short as 24 hours in chickens and as long as 6 days in waterfowl.

B. Clinical features.--Fowl plague is suspected in susceptible flocks when several birds are found dead without any signs except blue combs. The disease course is usually rapid in chickens, and the signs are a function of fever and edema. The early signs are general depression,

appearance of ecchymotic spots on the combs, wattle, and unfeathered parts of the legs. The infected birds are reluctant to move and inclined to hide in the dark areas of their pens. Dullness and inappetence are present. The feathers are ruffled with the neck retracted and, when moving, the birds stagger. Edema appears in the combs, wattles, and ear lobes and may also extend to the ventral parts of the head and around the eyes. Edema usually is accompanied by cyanosis of the comb and wattles which appear dark-red or bluish. The eyes are closed and the conjunctiva is congested, swollen, and petechiated. There may be slight puffing of the hock and metatarsal joints resulting from edema. The edematous parts of the body feel warm upon examination. Edema of the facial tissues and the epiglottis may interfere with respiration and affected chickens may open their beaks and gasp for air. Mucus may accumulate in the nasal cavities causing the birds to shake their heads and sneeze forcefully to expel the nasal discharge. Diarrhea may develop and finally the birds lie on their briskets with their head down; coma develops, and death usually occurs in 3-4 days. In certain cases, individual chickens may have convulsions immediately before death.

In turkeys, the disease is characterized by sudden onset of severe congestion of the non-feathered parts of the head, ruffled feathers, dullness in appearance, and slow movements. These signs are more dramatic in the males than in females. Later in the disease, the birds stagger in walking. Profuse white diarrhea is usually noticed. The course of the disease is 2-3 days longer than in the chicken.

The signs in ducks and geese are accompanied by fever, excessive thirst, and prone position. Signs include congestion of the beaks,

conjunctivae and non-feathered parts of the legs, listlessness, inappetence, and whitish-greenish diarrhea. Young birds frequently have convulsions, excitation, and rolling or circling movements. They may walk into fences and there may be occasional torticollis and ataxia. The hock joints are usually edematous and warm and diarrhea is very profuse and common.

III. Pathologic changes:

A. Gross lesions.--Chickens dying from a peracute form of the disease may not have any gross lesions. However, the changes usually found at necropsy are a result of fever and viremia. In the acute form, fully developed lesions may be characteristic when present. The conjunctivas are often congested and petechiated. The nostrils often show accumulation of thick, mucus, which is tinged with blood. Under the skin over the edematous parts of the comb, wattle, ear lobes, and other parts of the head, there may be infiltration of the intradermal and subcutaneous tissues with clear or sanguinous serous fluid. Congestion of the muscles is very noticeable. Hemorrhages that vary in size from that of a pinhead to an ecchymosis are on the surface of the peritoneal fat, peritoneum, serosal surface of the intestines, and heart as well as the dorsal surface of the sternum and pleural surfaces of the chest cavity. Petechial hemorrhages scattered as though they were sprayed over the area with an atomizer on the pleural surface of the sternum, over the epicardium, and in the vicinity of the coronary artery.

Hemorrhages or ecchymoses may be observed on the mucus surface of the proventriculus especially in the portion leading to the gizzard. Petechiae or ecchymoses may be seen in the gizzard surface under the cuticle. The serosal surface of the intestines may have petechia. The intestinal

mucosa may be covered with catarrhal exudate and hemorrhagic changes especially in the area of cecal tonsils. Blood vessels appear engorged, especially in carcasses which are examined soon after death. The lesions found in turkeys, ducks, and geese are similar to those of chickens but often are less severe.

B. Histopathological changes.--The main microscopic changes occurring in field cases were edema, hyperemia, hemorrhages, and foci of prevascular lymphoid cuffing, in myocardium, spleen, lung, brain, and wattles. Parenchymal degeneration and necrosis were present in the spleen, liver, and kidney.

IV. Diagnosis:

A. Field diagnosis.--Fowl plague is suspected when sudden deaths in a susceptible chicken flock are accompanied by edema, cyanosis of the head, hemorrhages in the proventriculus, gizzard, ventral surface of the sternum, coronary area, and fat of the abdominal cavity. However, such disease features are common to other disease conditions such as acute fowl cholera and velogenic Newcastle disease. Therefore, field diagnosis must be confirmed by virologic and serologic methods.

B. Laboratory diagnosis.--The preferred specimens for laboratory diagnosis are two or three carcasses of birds that were killed *in extremis* following the appearance of disease signs. Recent carcasses are also suitable. In the event difficulties are encountered in dispatching whole carcasses, specimens of liver, spleen, kidney, lung, trachea, and bone marrow may be shipped to the laboratory. All specimens should be frozen when they are received at the laboratory. The specimens should be identified properly and clearly and should be accompanied with a complete history

of the flock including any evidence of recent addition of birds to the flock.

The virus is readily isolated from the blood, liver, spleen, kidneys, lungs, and bone marrow. Embryonated chicken eggs, 9-11 days, are inoculated with bacteriologically sterile suspensions usually made from liver or spleen specimens. The amnio-allantoic fluid from dead embryos is then tested for ability to hemagglutinate chicken red blood cells. Inhibition of the hemagglutination reaction by a reference fowl plague antiserum and not by antisera prepared against the antigens of Newcastle disease of influenza A viruses indicates fowl plague infection. In addition, the infected amnio-allantoic fluids are used as an antigen in the virus neutralization test. Inhibition or the decrease of the death rate of embryos inoculated with mixtures of the antigen and fowl plague antiserum confirm the diagnosis of fowl plague virus. Young birds may also be given injections with the tissue extracts of field specimens or the fluids from the infected chicken embryos to prove the infectivity and degree of virulence of the isolated agent. In specimens from areas where fowl plague has not been reported, the isolated agent is identified by both the hemagglutination and the virus neutralization tests as well as by infectivity tests in young chickens. The combined results of the 3 tests will confirm the proper identification of the isolated agent.

C. Differential diagnosis.--Fowl plague may be confused with velogenic Newcastle disease, infections with influenza A viruses, acute fowl cholera and poisoning with phosphorus. Bacteriologic examination will eliminate fowl cholera, streptococcal infection, and pseudotuberculosis. Phosphorus poisoning produces hemorrhagic lesions, but it can be recognized

by the garlic-like odor of the intestinal contents, which also glow in the dark. The agar gel diffusion precipitin test may be used to differentiate fowl plague virus from Newcastle disease virus, but not from the influenza A group of viruses. The hemagglutination inhibition test, if used with limited numbers of test antigens or antisera, may lead to false negative results. The World Health Organization (WHO) has designated reference laboratories and provides them with a set of such antigens and antisera for preliminary identification of avian influenza viruses. The antigens and their antisera included are fowl plague Duck/England/'62, Turkey/Wisconsin/'66, Quail/Italy/1117/'65, Chicken/Scotland/'59, and Newcastle disease viruses.

V. Prognosis:

The prognosis is unfavorable. In natural outbreaks, the infected flocks may die within 7-10 days following the appearance of the signs. The remaining few survivors are immune.

VI. Epizootiology:

A. Geographical distribution.--Fowl plague is known in North Africa, Angola, Ethiopia, parts of Eastern Europe, Taiwan, Korea, and other regions of southeast Asia. In certain of these countries, mixed outbreaks of Newcastle disease and fowl plague occur.

B. Transmission.--Virus is found in all the fluids and tissues of the body and all the excretions and secretions of the sick or dead birds. Virus is very concentrated in the blood. Therefore, the diseased birds or their carcasses are major reservoirs of infection. The virus can remain viable in these carcasses for long periods. Introduction of one or more infected birds into susceptible flocks is followed by an outbreak within

3-7 days from the introduction. The virus may be transmitted directly by contact and indirectly by contaminated equipment as well as personnel who were exposed to infected birds. Infection also results from ingestion of virus with contaminated water or feed. Circumstantial evidence indicates that vectors may transmit the disease and that surviving birds may be temporary carriers and play a part in the spread of the disease.

C. Hosts.--Chickens and turkeys are most susceptible, but ducks, geese, pigeons, canaries, as well as sparrows, blackbirds, guinea fowl, and other wild birds also may contract the disease. Some mammals, including mice, rats, hamsters, guinea pigs, rabbits, ferrets, and monkeys can be infected experimentally.

VII. Control and eradication:

Addition of apparently healthy birds to a susceptible flock of poultry is one of the major factors in transmission of fowl plague. Therefore, recently acquired birds should be isolated and observed for three weeks. This isolation is essential when birds are imported from areas where fowl plague is known to occur. Dead birds should be submitted for diagnosis accompanied by a complete history.

The purchase of birds such as exotic species from enzootic areas is a means of introducing the disease into poultry flocks in this country. Therefore, all imported birds should be quarantined until it is determined that they are free from the disease. If fowl plague is introduced into a flock, depopulation of the sick and exposed birds is the most effective method of eradication. Dead birds should be placed in suitable containers to prevent the spread of the disease by direct and indirect methods. Infected premises should be quarantined and measures enforced to prevent the

movement of exposed birds or spread of infection by man or fomites. The infected premises should be depopulated and decontaminated thoroughly. The diseased birds should be destroyed by methods which are practical and insure inactivation of the virus. Restocking should not be undertaken for at least one month.

C. Immunization.--Various types of killed virus vaccines in general have not been effective. At least 2 vaccinations given a month apart are necessary. Some vaccines containing adjuvants, when inoculated intramuscularly may cause sterile abscesses in the muscle thereby reducing the meat quality. Vaccines containing live virus grown in chicken embryos or human cell culture have given satisfactory protection under experimental conditions. In 1962, a live virus vaccine was developed in Egypt from a virus isolated from a peafowl and was attenuated by passage in pigeon and chicken embryos. Field trials with this vaccine indicated that 80-90% of the vaccinated chickens were protected.

D. Treatment.--There is no known treatment for fowl plague.

VIII. Public health aspects:

There are no reports of infection of laboratory personnel working with fowl plague viruses. However, a virus closely related to fowl plague "Dutch Strain" was isolated from the blood of a person who was suffering from an undiagnosed illness subsequent to his return to this country from the Far and Middle East countries. Therefore, the public health significance of fowl plague infection needs further evaluation.

Fowl plague is a reportable disease in the United States. Signs of the disease should be reported immediately to disease control officials, such as the state veterinarian in the appropriate state or the county agricultural agent.

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LUMPY SKIN DISEASE *

I. Identification of the disease:

A. Definition.--Lumpy skin disease (LSD), also known as knopvelsiekte, exanthema nodularis bovis, and pseudourticaria, is an acute infection of cattle in Africa caused by a virus related to the pox group, characterized by the sudden eruption of intracutaneous nodules of varying size generally accompanied by acute local lymphadenitis and edema of the legs. Mortality seldom exceeds 10%.

B. Etiology.--Three or four groups of cytopathogenic agents associated with lumpy skin conditions have been isolated by means of tissue culture techniques. However, Neethling virus is now known to be the agent associated with true LSD. This virus is related to the pox virus group.

C. History.--The disease was first described as pseudourticaria of northern Rhodesian cattle in 1929. In 1949 the condition was observed in Transvaal and diagnosed in southern Rhodesia. From Rhodesia the disease spread rapidly, despite rigid quarantine measures. Sporadic outbreaks were reported in other regions of Africa. In South Africa outbreaks assumed epizootic proportions in 1962.

II. Signs:

A. Clinical features.--An initial rise in temperature with salivation, nasal discharge, and lameness is followed about 7 to 10 days later with an abrupt appearance of cutaneous nodules over the entire skin. These are firm and vary from 1 to 4 cm in diameter. Within a short time they become indurated and usually necrotic; later, the necrotic areas become ulcerative "sitfasts". There is a generalized lymphadenitis as the draining lymph nodes become edematous.

*Prepared by R. J. Yedloutschnig

B. Incubation period.--Under conditions of natural exposure this varies from 2 to 4 weeks.

III. Pathological changes:

A. Post mortem lesions.--Where the nodules extend to the mucosae of either or both the respiratory or digestive tracts, the animals usually die.

B. Microlesions.--Specific changes are demonstrated on tissue cultures inoculated with the virus. The histopathology of the nodules reveals edematous infiltration and perivascular proliferation of the mononuclear cells.

IV. Diagnosis:

A. In the field.--Appearance of distinctive lumps in bovine skin after an initial fever, with characteristic changes to the necrotic and ulcerative features arouse suspicion of LSD. However, it is characteristic of the disease that only part of a herd will be infected.

B. Laboratory.--Histological examination of nodule tissue and isolation of virus from excised nodules are methods commonly used. The agent is identified as Neethling virus by electron microscopy, characteristic cytopathogenicity in cell culture and by viral neutralization.

C. Differential diagnosis.--Since LSD may easily be confused with other maladies producing skin lesions, such as bovine herpes virus infections, fungal and bacterial conditions, laboratory confirmation is essential.

V. Prognosis:

The majority of animals recover spontaneously; the usual mortality rate is less than 2%.

VI. Epizootiology:

A. Geographic distribution.--At present the disease is confined to Africa.

B. Transmission.--The mode is obscure, but the rapid spread and ease of traversing long distances suggest the possibility of migrating birds or insects being involved.

C. Hosts.--Bovidae are the only known natural hosts.

VII. Control and eradication:

A. Preventive measures.--Strict quarantine and restrictions on movement of livestock have not succeeded in preventing spread of the disease in Africa. It is now believed that the control of potential insect vectors should be undertaken.

B. Sanitation and disinfection.--These measures are not necessary.

C. Treatment.--Supportive treatment and treatment of secondary infections are desirable.

D. Immunization.--Recovery of infection bestows a relatively short period of immunity (11 months). Tissue culture and egg-adapted vaccines have proven worthwhile.

VIII. Public health aspects:

There is no evidence that LSD can cause disease in man.

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Malignant Catarrhal Fever*

1. Identification of the disease:

A. Definition.--Malignant catarrhal fever (MCF), also known as snotsiekte, is an acute generalized disease of cattle and buffaloes, characterized by high fever, profuse nasal discharge, severe hyperemia, diffuse necrosis of oral and nasal mucosae, leukopenia, ophthalmia, corneal opacity and enlargement of lymph nodes. The clinical syndromes may be divided into 4 forms, namely the peracute, intestinal, head and eye, and mild. The naturally acquired disease is usually of the head and eye form and has low morbidity and mortality rates but high case fatality rates.

B. Etiology.--The etiologic agent of MCF in Africa is a herpesvirus with a capsid about 100 nm and an envelope about 140-220 nm in size. A similar agent may be the cause of MCF outside Africa because the disease in other continents resembles that seen in Africa in epidemiology, clinical signs, postmortem changes and histopathology. An etiologic agent of MCF has not yet been isolated from sick animals outside Africa.

The virulent African virus can be isolated from any tissue of the sick animal. But the highest titers are found in the buffy coat, lymph nodes and other tissues of the reticulo-endothelial (RE) system. This agent is cell associated and easily inactivated by either freezing or lyophilizations. It can, however, be preserved for about 2 weeks at 4 C or for months at -70 C in a medium containing at least 10% glycerol and 20% bovine serum.

*Prepared by M. Kalunda

Bovine thyroid cultures are so far the best medium for the isolation and propagation of the virulent strain. It produces focal cytopathic effect (CPE) marked by syncytial formation and vacuolation. Intranuclear inclusions may also be formed. The CPE becomes extensive on serial passages. The virulent virus is distinguishable from other herpesviruses of cattle by its pathogenicity and the absence of cell-free infectivity in cattle tissues or in early cell culture passages.

This virus is modified by cell culture passages to become cell-free and thus stable under various conditions, including freezing and lyophilization. The modified agent has a broader spectrum of infectivity in cell cultures. It produces CPE in thyroid cultures characterized by rounding of individual cells and detachment of affected cells from culture vessels. Cattle inoculated with this strain usually show the mild form of the disease rather that the head and eye form induced by the virulent virus.

C. History.--This disease is world-wide and usually occurs sporadically. However, epizootics of MCF that have involved large numbers of cattle and deer have been reported from widely separated parts of the world. The most recent epizootic of MCF occurred in cattle in Colorado during the winter of 1971-1972. In one group of 231 cattle, 87 (37%) died within 68 days.

The sheep associated disease was first reported in Europe in 1798; Switzerland, 1832; USA, 1920; and Canada, 1924. The wildebeest associated MCF was known to hunters in southern part of Africa during the early half of the 19th century. Their observation that this disease was acquired by cattle grazed with black wildebeest was reported in 1850.

The Boers named the disease snotsiekte in 1895.

II. Signs:

A. Clinical features.--The clinical picture of MCF is arbitarily divided into four forms, the peracute, intestinal, head and eye and mild forms. But, subdivision of the clinical manifestations into these forms has little practical use since there is considerable overlap in the syndrome observed. It does serve to emphasize, however, that the clinical syndrome can be quite variable and the diagnosis elusive.

(1) Peracute form: Severe inflammation of the oral and nasal mucosa and hemorrhagic gastro-enteritis are observed. The course of this form is 1-3 days.

(2) Intestinal form: This form is characterized by pyrexia, diarrhea, severe hyperemia of the oral and nasal mucosa. Nasal and ocular discharge as well as enlargement of lymph nodes are common features. The course of this form is 4-9 days.

(3) Head and eye form: This is the typical clinical syndrome of MCF. The first evidence of infection is pyrexia, often heralded 2-7 days earlier by nasal and ocular discharges. Bilateral nasal discharge begins as serous and soon becomes mucoid, mucopurulent and later purulent. Encrustation is common in late stages and causes partial or complete blockage of nostrils resulting in dyspnoea. At this stage the sick animal breathes through its mouth and usually shows drooling of saliva.

The oral mucosa exhibits intense hyperemia and diffuse superficial necrosis. Because the basal layer of the epithelium is rarely involved, the necrotic lesions are designated as erosions rather than ulcers. In the live animals, these lesions have a pink or red color due to exposure

of the underlying capillary bed. They are found on the lips, gums, hard and soft palate and the mucosa of the cheeks. The sharp-pointed buccal papillae are often involved and the tips slough leaving characteristic reddened blunted papillae. Petechiae of oral tissues are occasionally present. These changes cause severe pain and the animal objects to the examination of its mouth.

Changes in the eye include lacrimation that becomes purulent in late stages. Ophthalmia, prominent scleral veins and swollen eyelids are common features. Corneal opacity starts at the periphery and progresses centripetally resulting in either partial or complete blindness. Corneal opacity is usually bilateral but occasionally one eye is affected more severly than the other. Photophobia is usually associated with corneal opacity. An animal exhibiting this sign closes its eye most of the time and points its head away from the source of light.

Pyrexia is a common sign of the disease and is often biphasic. The temperature is usually high, 104-107 C, and remains high until shortly before death at which time, it is subnormal.

Increased thirst starts in early stages of the disease and continues until shortly before death. Anorexia is observed in the late stages of MCF. Constipitation is a common feature of the head and eye form but terminal diarrhea is occasionally observed.

Nervous signs are rare although shivering, incordinated movements and terminal nystagmus may be observed. Skin lesions are rare. The course of this form is usually 7-14 days.

(4) Mild forms: These are syndromes caused by experimental infection of cattle using modified viruses. They are often followed by recovery.

B. Incubation Period.--The incubation period of MCF under natural conditions is not known. The experimental incubation period varies from 9 days to 2 months, depending on the virulence of the virus used.

III. Pathological Changes.

A. Postmortem lesions.--The lesions vary according to the form and the course of the disease. Thus, animals that die of the peracute disease usually show no diagnostic changes. In this case the diagnosis must rest on the detection of the characteristic histopathological changes and positive results of transmission experiments.

In cases of the instestinal or head and eye form, the carcass may be normal, dehydrated or emaciated, depending on the course of the disease. The muzzle is often heavily encrusted and if wiped reveals an irregular raw surface.

The respiratory system may show minor or severe lesions. There may only be a slight serous or a copious mucopurulent discharge. When the course is short, the nasal mucosa shows congestion and slight to moderate serous exudate. Later, there is a profuse purulent discharge. The mucosa is then intensely congested and edematous. Erosions may be common. Occasionally croupous pseudomembranes form and if these are removed, raw surfaces remain. Turbinates are severely inflammed and often carry pseudomembraneous exudates. The pharyngeal and laryngeal mucosae are hyperemic, swollen and later develop multiple erosions or ulcers.

These lesions are often covered in part by a greyish-yellow exudate. The tracheo-bronchial mucosa is congested and usually petechiated; ulcerations may occur. The lungs are normal in peracute cases but may be emphysematous in others. Bronchopneumonia may complicate chronic cases.

The alimentary mucosa may show no significant lesions in the peracute disease. Hyperemia and diffuse superficial necrosis is a common feature in other forms of the disease. The erosive lesions often envolve the tips of buccal papillae, gingivae, both divisions of the palate and the cheeks. The tongue is often normal. The esophagus may show congestion, erosions and pseudomembranes. The rumen, reticulum and omasum may have areas of congestion but do not carry lesions. The abomasal mucosa is usually hyperemic, edematous and may carry petechiae. Hemorrhagic ulcerations are also common, especially in the pyloric region. The wall of the small intestine is firm and thickened by edema. The serosa may carry petechiae. The first half of intestinal mucosa may show severe congestion with blood-tinged contents. These changes decrease gradually towards the large intestine. Peyer's patches are usually normal but may show superficial necrosis. The large intestines often show minimal changes, mainly lines of congestion along the longitudinal mucosal rugae. Contents of the large intestine are scant and may be dry and pasty or stained with blood.

Characteristic lesions may appear on the kidneys. They are not always seen but when present are typical. They are usually small (2=4 mm) foci of nonsuppurative intestinal nephritis. These foci form slight rounded projections from the capsular surfaces. They are whitish and

represent infiltration of mononuclear cells. The urinary bladder is often normal or its mucosa may be congested.

The liver is slightly enlarged and may have miliary white foci. The gall bladder is distended but otherwise normal. The spleen is often enlarged and the Malpighian corpuscles are prominent. The heart may have petechiae on the coronary groove; the endocardium may show white patches.

All lymph nodes are usually affected but the abdominal ones are less consistently involved than those of the periphery, particularly those of the head and neck. Affected lymph glands are many times the normal size, usually 2-5 times (but occasionally up to 10 times) and are usually hemorrhagic. Some (including hemolymph nodes) are usually too small to recognize, but under the influence of the disease, becomes quite obvious.

B. Microlesions.--Confirmation of MCF and differentiation from similar diseases are based on the histopathological changes. These changes are pathognomonic and are found in lymphoid tissues and in the adventitia and walls of small blood vessels in any organ. They are the same in rabbits to which the disease can be transmitted.

The vascular lesions take the form of a fibrinoid necrotizing vasculitis and cellular accumulation in the adventitia. These lesions are focal and are seen in all cases of MCF ragardless of the form of disease.

IV. Diagnosis.

A. In the field.--The history of the disease indicating close contact between the infected animal and calving wildebeests in Africa or lambing ewes elsewhere, aids a tentative diagnosis. The long incubation period of this disease, however, often shadows the association between the natural and alien hosts of MCF. Typical clinical features help in

forming a presumptive diagnosis. These include high temperature, profuse nasal discharge, severe congestion and diffuse necrosis of oral and nasal mucosae, ophthalmia, corneal opacity and gross enlargement of peropheral lymph nodes. One or more animals in a herd are usually affected in the normal pattern of MCF.

B. Laboratory diagnosis.--Specimens required for laboratory examinations in the study of MCF are:

(1) Blood for virus isolation and cell counts. Blood (about 200 ml)should be collected in EDTA (1 mg of EDTA per 1 ml of blood) or heparin.

(2) Tissues for virus isolation. Spleen, lymph nodes, adrenals, and thyroids are suitable for virus isolation.

Blood and tissues for virus isolation should be refrigerated but not frozen and should be sent to the laboratory as soon as possible. Buffy coat or cell suspensions from these tissues are inoculated onto established thyroid cultures. These are checked for the typical CPE that may be formed 4-20 days after inoculation. Primary cultures of thyroid, adrenal, kidney and tests cells from infected animals show the typical CPE. But no CPE is observed in primary cultures of buffy coat or lymph nodes from the same animal, although these tissues have the highest titers.

(3) Tissues for histopathological studies. Thin slices of kidney, spleen, liver, adrenals and lymph nodes are fixed in 10% formalin in physiological saline or PBS.

(4) Serum for serological tests. Paired serums are required, the first collected at the onset of the disease and the second during convalescence.

C. Differential diagnosis.--The clinical syndrome of MCF resembles that of other diseases especially those that cause necrosis, ulcerations and erosions of the oral mucosa of cattle. Differential diagnosis should therefore include bluetongue, bovine viral diarrhea-mucosal disease (BVD-MD), rinderpest, vesicular diseases and ingestion of caustic substances.

(1) Bluetongue: The clinical reactions of MCF resemble bluetongue especially in the diffuse necrosis of oral mucosa and crusting of the muzzle. Lameness common in bluetongue is absent in MCF and ophthalmia and corneal opacity often associated with MCF are rare in bluetongue.

Virological, serological and histopathological examinations are essential for differential diagnosis of these diseases.

(2) BVD-MD: The classic clinical syndrome of BVD-MD occurs sporadically and is characterized by fever, leukopenia, diarrhea, lacrimation, nasal discharge and erosions of the oral mucosa.

Oral lesions in this disease, unlike those of MCF, are discrete, rounded or linear depressions. Severe hyperemia and ophthalmia, common in MCF are not observed in BVD-MD. Diarrhea is also rate in MCF.

Final differential diagnosis requires virological, serological and histopathological tests.

(3) Rinderpest: Rinderpest, enzootic in Africa and parts of Asia is exotic in this country. The clinical syndrome of rinderpest is similar to that of BVD-MD. The introduction of rinderpest virus into the highly susceptible bovine population of USA would result in high morbidity and mortality rates, rapid transmission between animals and herds and a disease generally more drastic than that of MCF. Mild strains

of rinderpest virus could easily be misdiagnosed as the mild form of MCF.

(4) Vesicular diseases: i.e., FMD and vesicular stomatitis are excluded on the ground that these diseases elicit vesicles on the oral mucosae, teats, and coronary bands of cattle. These vesicles rupture quickly leaving flaps of epithelium.

V. Prognosis:

Prognosis is poor for MCF. The case fatality rate of MCF is 90-100% in Africa while that of a major Colorado epizootic of MCF was 30-40%.

VI. Epizootiology:

A. Geographical Distribution.--MCF has been reported in many countries all over the world.

B. Transmission.--This disease is transmitted from the natural reservoirs to cattle, the alien host. Blue wildebeest (Connochaetes taurinus) and the black wildebeest (Connochaetes gnu) are two of the natural hosts of the MCF virus in Africa. They are inapparently infected. Sheep may be the natural carriers of a similar virus in other continents. In the natural disease transmission may occur when cattle graze with calving wildebeest or are housed with lambing ewes. Close contact between the donor and the recipient is presumed to be essential factor in transmission. Transmission of MCF between sick and other susceptible cattle has never been reported.

The mode of transmission and the association of calving wildebeest or lambing ewes with the infection of this disease have not yet been defined. The latest studies conducted independently on cattle at this laboratory and on wildebeest in Africa have shown that in both cases

nasal discharges of infected animals carry the MCF virus. This finding partly explains how cattle could acquire the infection by grazing with wildebeest and indicates that under certain conditions the disease could be transmitted among cattle by close contact.

C. Host Range.--Infection with the virus and clinical signs of MCF have been reported in the following species: cattle, buffaloes, Pere David's deer, and rabbits. Preliminary studies at this laboratory have shown for the first time that sheep can be infected with the wildebeest strain of MCF virus.

VII. Control and Eradication.

A. Preventive measures.--Our present knowledge of MCF dictates that the incidence of this disease can be reduced by separating cattle and the natural hosts during the lambing or calving seasons.

B. Natural Immunity.--Cattle or rabbits recovered from this disease develop solid immunity against all strains of MCF virus.

C. Induced Immunity.--All strains of the wildebeest MCF virus are homologous by the virus neutralization (VN) test. No effective vaccine is available for this disease.

VIII. Public Health Aspects.

The disease is not transmissible to man.

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NEWCASTLE DISEASE*

I. Identification of the disease:

A. Definition.--Newcastle disease (ND) is also known as pseudoplague of fowl, pseudofowl pest velogenic viscerotropic Newcastle disease (VVND), and Ranskeldisease. It is a highly contagious and destructive viral disease which attacks chiefly chickens and turkeys. Other poultry and feral birds as well as man may contract the infection.

B. Etiology.--Newcastle disease is caused by a ribonucleic acid (RNA) paramyxovirus of a size estimated to be 80 to 180 nm. The virus has the ability to agglutinate and lyse chicken erythrocytes (among those of other species), a characteristic which may be inhibited by ND specific antiserum. The virus may be inactivated in 1 minute at 100 C and in 30 minutes at 60 C. At 37 C NDV activity recedes in a matter of days. The virus is destroyed by ultraviolet light as well as several chemical disinfectants, among these formalin (0.5%) inactivates the virus in approximately 18 hours.

Although the virus is adversely affected by warm temperature and solar radiation, it may be protected from these influences by its proteinacious coating. Therefore cleaning and sanitization of contaminated surfaces and objects before application of disinfectants facilitates their effectiveness.

Newcastle disease strains are further classified on the basis of their virulence into velogenic, mesogenic, and lentogenic strains. Velogenic strains are exotic to poultry flocks in the USA, but make incursions into the country occasionally.

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*With particular reference to exotic forms. Prepared by A. H. Dardiri

C. History.--The disease was first described by Kraneveld in Java (1926) followed by the report of Doyle in 1927 of the malady in a flock of chickens in Newcastle, England. By 1940 ND was reported from the Philippines, Asia, Australia, and Africa; it was later reported from the continent of Europe. The initial form of the disease was characterized by extensive hemorrhage of the digestive tract. Other characteristics caused confusion with fowl plague at times. In 1944 ND was identified in California and then on the East Coast of the USA. Through the years there have been outbreaks in many poultry-raising areas of the Western Hemisphere. Usually the disease encountered was mild and accompanied by respiratory and neurological disorders which lead to the term "pneumoencephalitis" being applied.

On several occasions a more severe form of the disease appeared in the USA, mainly through the importation of exotic birds. The most recent incident was the 1972 outbreak in California which required a costly program of vaccination and "stamping out" procedures to effect eradication. This form of the disease was referred to as velogenic viscerotropic Newcastle disease (VVND). Approximately 40 million chickens were destroyed and the approximate cost of eradication was 58 million dollars.

II. Signs:

A. Clinical features.--An outbreak of VVND may be so acute and severe as to kill nearly all of a flock within 3 or 4 days. In the peracute form the birds die suddenly without noticeable signs. More often in the acute form, the birds first appear listless, their respiratory rate increases, a pyrexia appears, weakness becomes apparent, followed by prostration and death in 5 to 7 days. The sick birds may display watery, greenish diarrhea which is sometimes blood stained and profuse. As a result of the fever and

diarrhea, the birds appear dehydrated. A rise of 4-6 C may occur early and then subside to below normal levels before death. Clonic spasms and torticollis may appear in birds that survive. Mortality is usually 90% to 100%.

Frequently some chickens show cyanosis of the comb and wattles and edema of these organs as well as the area extending from between the wattles and the upper part of the neck. There is considerable variability in the severity of signs. This variation is influenced by the species, age, and natural resistance of the birds as well as the potency of the viral strain.

B. Incubation period.--The incubation period is usually 2 to 6 days but may be as short as 2 or 3 days.

III. Pathologic changes:

A. Post-mortem lesions.--The mouth usually contains mucus discharges which may be tinged with blood. Dark cyanotic combs may be found on birds at death. Facial and neck edema may be severe, especially in young birds. Straw-colored exudate may be excreted from the eyes and nasal openings. A diphtheritic pharyngitis may be present. Occasionally edema is present in the subcutaneous tissue of the face, entrance of the thorax, or at the end of the keel. The tracheal lesions are usually hemorrhagic without free blood in the lumen of the trachea. Occasionally the lining of the proventriculus is hemorrhagic as well as the serosal surface of the organ. Upon removal of the lining, the surface of the gizzard may be found hemorrhagic. Numerous small hemorrhages are frequently found in the intestine.

The most constant necropsy finding in velogenic Newcastle disease is occurrence of hemorrhagic lymphoid foci in the intestines. These occur

in the duodenal end and also prominently in the cecal tonsils. Lymphoid plaques or patches may be seen protruding on the surface of the intestinal wall. The large intestine and cloaca may have necrotic foci. Excessive yolk-like fluid is often observed in the abdominal cavity of laying hens.

B. Microlesions.--Microscopic necrotic lesions of the spleen, liver, gall bladder, intestines, and heart often characterize velogenic Newcastle infections. Inflammation and cellular infiltration characterize the serosal lining of the thoracic and abdominal cavities. The hemorrhagic and necrotic lesions of the intestines often consist of lymphoid aggregates. The lesions in the proventriculus are associated with minute ulcerative changes. Lymphocytic infiltration has been described in the pancreas.

IV. Diagnosis:

A. In the field.--The signs of VVND and course of the disease closely resemble those of a number of avian diseases including fowl plague, laryngotracheitis, and the diphtheritic form of fowl pox. Laboratory confirmation of a diagnosis is therefore mandatory.

B. Laboratory.--The surest method of diagnosing VVND or ND is the isolation and identification of the causative virus. Specimens for attempting viral isolation should be selected from cases in the early or even the prodromal stages of the disease. The viral strains causing VVND are widely distributed in the avian body but are present in greater concentration in particular tissues such as the liver, spleen, blood, and lungs. The virus may be isolated by inoculation of these tissue triturates into 9-11 day embryonated chicken eggs. After proper incubation the virus will be found concentrated in the chorioallantoic (CA) fluids. Clear, bacteria-free CA fluid is then tested for avian erythrocyte agglutinating activity. It

should be determined if the reaction is inhibited by known ND antiserums. Identification of NDV is accomplished by means of serum neutralization tests in embryonated chicken eggs, also.

C. Differential diagnosis.--Velogenic viscerotropic ND is likely to be confused with fowl plague (FP). In FP the hemorrhagic lesions on the mucosa of the proventriculus and beneath the proventricular cuticula are much more extensive and severe than in VVND. Virulent NDV kills pigeons in from 3 to 5 days when inoculated intramuscularly. In contrast, the virus of FP, when inoculated into pigeons in the same manner will not cause signs or death. The virus of ND is not neutralized by the antisera from other avian diseases.

V. Prognosis:

In susceptible chickens VVND causes high mortality.

VI. Epizootiology:

A. Geographical distribution.--Velogenic viscerotropic ND has become a menace to the poultry industry. The disease is endemic in India, Indochina, the Philippine Islands, Japan, Korea, Ceylon, Kenya, Egypt, Israel, Syria, and other countries. High mortality has characterized epizootics in these countries. Recently, this type of disease has been reported from countries in various parts of the world. The sporadic outbreaks of VVND in the USA recently were traced to the importation of exotic species of birds (such as parrots and myna birds) from the Orient. These foci of disease were successfully eradicated but not before the disease had spread to commercial poultry flocks in southern California, where the disease was widely spread in several counties.

The disease there was eradicated by the "stamping out" method, disinfection, and decontamination as well as by vaccination. As stated earlier the cost of the campaign in California was approximately 58 million dollars; about 40 million dollars worth of chickens were destroyed.

B. Transmission.--Velogenic viscerotropic ND is transmitted within a susceptible flock by aerosol, contact, contaminated feed, and frequently by people, such as the flock attendants. Visitors are known to have transmitted the disease from one flock to another.

Dissemination of the virus between flocks over long distances has been frequently related to movement of apparently healthy birds in the prodromal or recovery stages. The disease may be transmitted through feeding of infected offal, feed, or water. Contaminated fomites, such as crates, sacks, trucks, etc., may act as mechanical carriers.

The rapid transportation afforded by the airplane has been responsible for spread of the disease. Myna birds shipped from Indochina to Florida and California resulted in outbreaks of VVND.

Virus has been recovered from dressed poultry and this may be another factor responsible for the spread of the disease from one country to another.

C. Hosts.--Domestic fowl and turkeys are the chief hosts although other species of birds are susceptible. During natural outbreaks guinea fowl, ducks, geese, pheasants, partridges, parrots, and myna birds, as well as other flying species, were involved. There is lack of agreement among investigators regarding the susceptibility of wild birds, due to lack of research data regarding many phases of virus-host relationships in these species. However, it is known that most of the gallinaceous

species that are free from antibodies will suffer a lethal infection when inoculated with NDV.

VII. Control and eradication:

A. Preventive measures.--In addition to vaccination, every effort should be exerted to avoid the introduction of the virus to countries which are free from it.

B. Sanitation and disinfection.--A high standard of sanitation should be practiced in management of poultry flocks and on poultry farms. Particular attention should be given to eradication of flies, rodents, and accumulations of feral birds. Upon diagnosis of VVND, the infected premises should be quarantined, the affected flocks and all other birds on the premises should be depopulated and rendered. All the premises should be thoroughly cleaned and disinfected (with a reliable chemical, such as orthophenylphenate). The premises should remain depopulated for at least 30 days before introduction of replacement birds.

The use of sentinel birds on the infected premises for one month before introducing a new flock is useful to indicate the presence or absence of VVND.

C. Treatment.--No drug treatment is known to cure birds from ND infection.

D. Immunization.--Two types of vaccine are available commercially, namely, killed and live virus. These vaccines have been widely used and are very effective when used under favorable or ideal laboratory conditions and according to the manufacturers' specifications. But their efficiency may be modified by conditions in the field, including age, resistance, laying stresses, and sanitary conditions of the flock. In all vaccination

programs the duration of immunity does not exceed several months and may be as short as 3 weeks. Two important aspects regarding vaccination are: (1) There is great variation in protection afforded individual birds by any vaccination; (2) the immunity of individual birds will be affected by the stresses to which they are exposed by management, sanitation, and husbandry of the flock.

VII. Public health aspects:

Newcastle disease virus is capable of causing conjunctivitis in man. Infection is acquired by contact with virus in the laboratory through handling live virus, or in the field by the administering of a live vaccine. Although the majority of human infections are localized in the eye, in a few instances, infection has been accompanied by mild headaches and muscular pains; the virus has been isolated from urine. Laboratory diagnostic workers and others who work with the live virus, including vaccines, should be aware of the possibility of contracting the infection.

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PESTE DES PETITS RUMINANTS *

I. Identification of the disease:

A. Definition.--Peste des petits ruminants (PPR), also known as pseudorinderpest of small ruminants, pest of sheep and goats and kata, is an acute or subacute virus disease of goats and sheep. The acute form is characterized by necrotic stomatitis and intestinal and lymphoid tissue syndromes which resemble those of rinderpest virus (RV) in cattle. The virus is closely related antigenically and immunologically to RV. Cattle are not clinically affected. Goats are more susceptible than sheep and mortality rates in goats vary with activation of latent infection with bacteria or protozoal parasites.

B. Etiology.--The virus is identical to RV in its physical, chemical, and general antigenic properties. The virus particles are spherical in shape and have a diameter of 5,000-7,000A° which is larger than RV. The virus particle consists of a core of ribonucleic acid within a fringed outer membrane. Accordingly, it is classified as a member of the paramyxovirus group. The virus has been adapted to multiply in ovine embryo kidney cell culture and induces the formation of large size multinucleated cells with large eosinophilic inclusion bodies. The PPR virus in tissue culture maintained at 40 C retains its pathogenicity for goats and can be used to immunize cattle against experimental RV infection. Tissue culture virus maintained at 37 C rapidly loses its virulence for goats and immunizes this species against virulent virus.

C. History.--Peste des petits ruminants has been reported since 1942 in sheep and goats in some of the ex-French territories of West Africa. The etiological viral agent was isolated and identified in 1956

*Prepared by A. H. Dardiri

as a strain of RV which was naturally adapted to and pathogenic for goats and sheep. It was attenuated for cattle and was believed to lose its ability to infect them by contact. Shortly afterwards extensive new epizootics appeared in Nigeria, causing death among Plateau dwarf goat and sheep. Later the virus was isolated in East Africa from naturally infected goats. Kata is a name used locally in Western Nigeria for pseudo-rinderpest of goats. The disease was first recognized in goats in 1965 and its viral etiology was established in 1968. Goats from Mambilla Plateau in Nigeria were susceptible to experimental infection with either Kata or PPR and disease signs, and gross or histopathological lesions were indistinguishable. The PPR failed to provoke clinical signs of illness in goats that had recovered from Kata. Caprinized RV infections failed to cause clinical reactions in goats previously exposed to Kata and PPR strains. These findings lend support to the conclusion that PPR and Kata viruses and RV are antigenically related.

II. Signs:

A. Clinical features.--The acute form is accompanied by a sudden rise of temperature to 104-106 F. The affected animals appear ill and are restless. They have a dull coat, dry muzzle, depressed appetite, congested mucous membrane, and serous nasal discharge. Ulceration in the buccal cavity sometimes occurs. However, involvement may be limited to severe congestion of the laryngo-pharyngeal mucous membranes. The disease course is relatively short, about 8-10 days, usually resulting in death.

In the subacute form of the disease, which is the form usually seen in experimentally infected animals, no clinical signs of disease

are evident for about 5 days after infection. On about the 6th day, fever and a serous nasal discharge may be found. The fever reaches its peak after 2 or 3 days and then falls after about a week with the onset of diarrhea. In fatal cases, diarrhea becomes progressively more severe, followed by dehydration, emaciation, and prostration. The hind quarters and tail become soiled.

Nasal and lacrimal discharge usually begin as clear serous exudates that later become mucopurulent. On about the 7th to 9th day after inoculation, superficial erosions of the lip and buccal mucosa may be observed. Similar ulcerations or erosions may be found in females on the labial surface of the vulva. The sites of predilection for ulcerations are the lips, gums, buccal papillae, and the ventral surface of the tongue. Stomatitis may or may not be accompanied by salivation. Affected animals may have profuse thick mucoid nasal discharge with coughing and sneezing. Some animals may develop excoriation of the muzzle and commissures of the mouth. Most goats die 6-12 days after the rise in temperature. Some may linger on for 3 weeks after onset of illness. The most frequent complications are secondary bacterial infections resulting in pneumonia and broncho-pneumonia. Pasteurella and Mycoplasma organisms are frequently recovered from such eases. Infection with PPR may activate latent intestinal coccidial infection as well as hematophagous parasites. Abortions occur frequently.

B. Incubation period.--The incubation period in the natural disease may range from 2-15 days.

III. Pathologic changes:

A. Post-mortem lesions.--Animals that die following an acute form of PPR do not exhibit lesions other than congestion of the mucous membranes

and occasional congestion of the ileocecal valve. In some cases, there may be secondary broncho-pneumonia. Lesions in frank clinical cases resemble those of RV infection in cattle, but tend to be less intense. Pulmonary involvement is more frequent than in RV infections. The carcass is emaciated, soiled, fetid, and the nostrils are encrusted by a purulent nasal discharge. The lips are hyperemic. Lesions in the mouth vary from a few erosions of the mucosa of the soft palate to extensive necrotic ulcerative stomatitis. Erosions may extend into the congested pharynx. The mucosa of the abomasum may show a diffuse congestion. Severe congestion may extend throughout the alimentary tract but, more often, changes are limited to the duodenum, ileum, cecum, and the upper colon. The mucosa of the ileocecal valve is a prominent site of congestion and sometimes there may be hemorrhage. The crests of the longitudinal folds of the cecum, colon, and rectum are sometimes congested giving the appearance of "zebra" stripes.

The mucosal lining of the upper respiratory tract and trachea is generally congested. Patches of congestion in the lungs is common, and on occasion, broncho-pneumonia lesions will affect the apical and cardiac lobes.

Usually the heart appears grossly normal. Sometimes, petechiae are present near the coronary vessels. Most usually, the mesenteric lymph nodes are edematous. The spleen usually is grossly normal, but occasionally it is swollen slightly and the capsule is injected. Congestion and erosion is common in the large Peyer's patches of the terminal part of the ileum. There are no specific liver lesions.

The mucous membrane of the urinary bladder is normal or slightly congested. Kidney congestion and erosion of the vulvar and vaginal mucosa occur occasionally.

B. Microlesions.--The necrosis of the mucosa of the oral cavity is marked by the presence of intranuclear and intracytoplasmic inclusion bodies and occasional syncytia in the stratified epithelium as in RP. Intranuclear inclusion bodies are found in the reticulo-endothelial cells close to the sinus and germinal centers of the lymph nodes.

IV. Diagnosis*:

A. In the field.--A presumptive diagnosis of PPR may be made when there is a new epizootic in goats and sheep which is accompanied by mortality and disease signs and lesions as described for PPR. However, because some of these signs or lesions are common to other sheep and goat diseases, a laboratory confirmatory diagnosis is necessary, which is achieved by isolation and identification of the virus.

B. Laboratory.--Blood, spleen, and mesenteric lymph nodes from sick animals or those in extremis are the tissues of choice for submission to the laboratory. Serums from recovered animals are necessary for the detection of PPR antibodies. Among the tests, which may be performed in the laboratory, is the demonstration of cross-protection of goat and sheep infected with PPR and RV. Susceptible goats exhibit PPR disease signs upon inoculation with the virus. Cattle resist challenge with the virulent RV subsequent to their inoculation with PPR virus.

The PPR virus can be isolated in cell culture and identified using serological tests such as virus neutralizing and complement-fixation tests.

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*See also appendices 1 and 2.

Antibodies in the serums from animals which recovered from PPR may be detected and assayed by the same serological tests.

C. Differential diagnosis.--Many types of secondary pathogens are involved; however, the more common are protozoa, either in the circulatory or the intestinal systems. Dual infection yields complex clinical signs and postmortem lesions. Often the resulting disease features are dominated by those attributed to activated infections. Diseases such as bluetongue, coccidiosis, Nairobi sheep disease, contagious ecthyma, plant and mineral poisoning have clinical and pathological manifestations similar to those of PPR. The diagnosis of bluetongue requires serological confirmation. The only certain way to diagnose coccidiosis is to find active lesions containing coccidia at necropsy. Sometimes the history of an outbreak may help differentiate PPR from plant and mineral poisoning; however, serological tests are necessary. Mice inoculated with blood from sheep with Nairobi sheep disease die, but they will remain healthy if they are infected with PPR.

The buccal lesions around the mouth and nasal openings associated with PPR can be confused with those of contagious ecthyma and pox. Therefore, laboratory confirmation is necessary for disease differentiation. Inoculation of susceptible animals and histopathological examination will assist in diagnosis. In sheep and goat pox, papules, vesicles, and scab formation are generally more extensive on the body. Contagious ecthyma produces proliferative type lesions and can be distinguished by cross-immunity tests.

V. Prognosis:

Mortality in goats range from 10-90%. Sheep are less susceptible than goats and rate of recovery is significantly higher than in goats. The mortality rate is affected by the animal's condition, its innate resistance, the virulence of the virus, and secondary complications caused by activation of latent infection in the animal.

VI. Epizootiology:

A. Geographical distribution: PPR is an enzootic disease in West French Africa. It was reported mainly in the southern and central regions of these countries and rarely in the northern regions. The disease exists in Nigeria, the Ivory Coast, Senegal, and Dahomey.

B. Transmission.--Infection may be transmitted to susceptible animals by direct contact with infected animals or indirectly by contact with secretions and excretions. Movement of infected animals may be the chief factor in the spread of the disease. All tissues and fluids from infected animals should be considered infectious throughout the period of clinical illness.

C. Hosts.--PPR is an enzootic disease of goats and sheep in West Africa. Cattle develop serological response without clinical signs as a result of inoculation. Infection of cattle by contact has not been conclusively proven.

In spite of the close resemblance of PPR disease signs, lesions and the viral properties to RV, cattle exposed to PPR do not have any clinical reaction but do acquire a solid immunity to RV.

VI. Control and eradication:

A. Preventive measures.--When PPR is suspected, state and federal veterinarians should be notified. Methods that are applied to RV eradication are useful in eradication and control of PPR. The stamping out procedure is recommended when the disease appears in new areas. All sick goats and sheep and those in contact should be slaughtered and disposed of by burning, burying, or rendering. Infected premises should be decontaminated and the area should be quarantined. Import restrictions on sheep and goats from countries where the disease is enzootic should be applied. Movement of animals and their products from suspected premises should be prevented. Epizootic surveillance of sheep, goats, and cattle should also be undertaken to determine the extent of disease present or its dissemination.

B. Treatment.--There is no specific treatment for PPR. However, administration of products which alleviate bacterial and parasitic complications decrease the mortality of affected herds.

C. Immunization.--Susceptible goats have been protected from infection with PPR by inoculation with serum from cattle which were hyperimmunized against RV. Limited success in protection of susceptible animals was obtained by using inactivated and lapinized RV vaccines. More recently, the use of cell culture RV vaccine has proved effective in the immunization of susceptible goats against exposure to natural infection or experimental immunity challenged with PPR virus. In addition, an attenuated vaccine was obtained by 51 passages of PPR virus in embryo kidney cell culture. This vaccine is very efficient in protecting goats and sheep from natural disease for about a year.

VII. Public health aspects:

Man is not susceptible to RV and it is believed that PPR is not communicable to human beings. However, serum PPR convalescent goats inhibit measles virus in the hemagglutination inhibition test.

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APPENDIX 1

SUMMARY OF CHARACTERISTICS OF PESTE DES PETITS RUMINANTS (PPR)

Description	Cause and Distribution	Host	Pathology	Diagnosis
An acute, sub-acute or chronic disease of goats and sheep with rinderpest-like lesions in cattle. Necrotic ulcerative stomatitis, pneumonia and diarrhea.	Virus. Ex-French territories in Agrica.	Goat, sheep and cattle.	Ulcerative necrotic stomatitis. Congestion in the alimentary tract, diarrhea and emaciation.	History, signs and lesions, virus isolation and identification. Gross protection tests in goats and cattle.

Incubation Period	Mode of Transmission	Period of Communicability	Control Measures
6-15 days.	Direct contact with sick animals. Contamination of premises by secretions of sick animals.	During incubation period and disease course.	Stamping out and quarantine measures. Use of vaccine in enzootic areas.

APPENDIX 2

DIAGNOSTIC TESTS FOR PESTE DES PETITS RUMINANTS (PPR)

Microbiological Procedures	Histological Examination	Serological Tests	Animal Inoculation
Virus isolated from spleen, blood and lymph nodes	Nasal catarrh, ulcerative and necrotic stomatitis, pneumonia and congestion of alimentary tract	Virus neutralization and complement fixation tests	Susceptible goat, sheep and cattle

RINDERPEST *

Introduction:

The recorded history of rinderpest dates back to before and during the early centuries of the Christian era. In the second half of the fourth century, it was recognized as a distinct clinical entity. Outbreaks in Europe frequently came from the East and were believed to have originated in the area of the Caspian Sea and the adjacent steppes. Outbreaks of rinderpest invariably followed in the wake of major military campaigns.

I. Definition of the disease and its etiology:

Rinderpest is an acute, sometimes subacute, febrile disease of ruminants and pigs, characterized by severe congestion, hemorrhage, and erosion of the mucus membrane of the alimentary tract. Inapparent infections can occur, but the disease is usually accompanied by severe diarrhea.

The disease is caused by a virus having a diameter of approximately 12 mμ. Tentatively, it has been classified as a myxovirus.

II. Hosts: natural and experimental:

Ruminants and pigs are the principal natural hosts of rinderpest, but other wild animals have been shown to be natural hosts. The experimental host range includes goats, sheep, rabbits, hamsters, guinea pigs, embryonated eggs, young chickens, and young mice. Dogs, ferrets, and monkeys have been inoculated, experimentally, but their susceptibility to the virus has not been definitely established. However, cross neutralization of sera from these hosts inoculated with rinderpest, canine distemper, and measles viruses, respectively, has been reported.

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*Prepared by C. J. DeBoer

III. Epidemiology:

Epizootiologically, rinderpest is a comparatively simple disease. Transmission takes place primarily between sick and healthy animals. The virus may be transmitted by contact with secretions and excretions of infected animals. The available evidence indicates that persistent carriers are rare. Excretion of virus from sick animals is usually limited to 2-3 weeks. However, additional studies on this particular aspect of the disease would be very valuable. Contaminated grass, soils, and water might contribute to transmitting the infectious agent, but the danger from these sources appears to have been over-estimated. The greatest dangers are associated with movements of ruminants and pigs out of enzootic areas into areas free of the disease.

Virgin epizootics in low-risk areas are usually explosive and are characterized by high morbidity and high mortality in animals of all ages. Epizootics in high-risk areas are often less severe because of the innate resistance of livestock in the neighboring areas.

IV. World situation:

With the exception of parts of Turkey, Europe has been reported to be free of the disease since 1930, largely due to slaughter and strict enforcement of other control measures. However, rinderpest has continued to spread to wide areas of the African continent, the Middle East, Pakistan, India, Burma, and Southeast Asia. At present, little is known concerning the status of the disease in China. Although no widespread outbreaks have been reported from Indonesia, it is quite possible that occasional outbreaks do occur. Both North and South America have remained free of the disease except for one outbreak of short duration, in Brazil, in 1921.

Following World War II, small outbreaks have been reported in Rome and Trieste in 1951 and 1954, respectively. In both instances, the outbreaks were confined to primary foci and involved animals imported from enzootic areas destined for zoological gardens.

V. Diagnosis:

A. Field.--Cases of rinderpest confirming to the classical description have become rarer, with increases in the immunity cover of herds through frequent or regular mass vaccinations, and wide clinical variations may be encountered. The incidence of the various clinical forms appear to be related to the innate resistance of the infected animals and the degree of virulence of the virus causing the infection. A high incidence of frank clinical cases may be expected only when outbreaks occur in areas that have been free of the disease for extensive periods of time, thus having an animal population of low innate resistance. Many local breeds and species of animals possess a high innate resistance to rinderpest and their infection results in a vague subclinical illness that seldom arouses suspicion.

What to look for in suspected field cases: (See attachment.)

Some diseases, such as virus diarrhea, mucosal disease, and parasitic gastro-enteritis are difficult to distinguish from rinderpest. Final diagnosis of such cases requires laboratory support (Dr. Dardiri will discuss the clinical signs to look for in the field and at quarantine stations).

B. Laboratory.--Excellent laboratory techniques are available for the diagnosis of rinderpest and differentiation from other suspected diseases. The possibility of having rinderpest infections in a country that has been free of the disease for a long time and, particularly, in a country where the disease had never existed, is a serious matter. In a diagnosis

the fulfillment of Koch's postulate should be required. These are:

1. Isolation and identification of the virus.
2. Reproduction of the disease in normal animals with the virus isolated from the field specimen.
3. Challenging of any survivors inoculated with the field specimen using known virulent rinderpest virus.
4. Test for rinderpest antibodies in sera from field cases or the animals on test for demonstrating virus may be made with the following laboratory procedures:
   (a) Complement fixation
   (b) Agar gel diffusion precipitation
   (c) Neutralization in animals or tissue cultures

C. Similar disease syndromes.--Syndromes similar to that of rinderpest may be encountered in cattle, sheep, and pigs. In cattle, for example, anthrax, foot-and-mouth disease, vesicular stomatitis, ephemeral fever, malignant catarrah, rhinotracheitis, and others, could be present.

D. Selection, collection, identification, packaging, shipping, and transmittal of samples.--Obtain a blood specimen from an infected or suspected animal. If the animal is necropsied, portions of the spleen and lymphoid tissues, particularly the mesenteric lymph nodes, should be obtained and placed in sterile screw-capped glass containers, properly labeled and identified. Tissue specimens should be shipped in especially designed styrofoam dry-ice containers. Whole blood may be packed and shipped in wet ice, whereas sera should, preferably, be frozen in dry ice for shipment. It is important that surgical adhesive tape, rather than paper labels be used since the former have been found to adhere firmly to the containers

under various conditions. Containers for shipping tissue specimens as well as serum vials, should not be filled to capacity; space should be left for expansion during freezing. Currently, plastic containers of various sizes and shapes have become available and inasmuch as they are unbreakable, are superior to the glass containers.

Finally, a complete and comprehensive description and all pertinent information relative to the samples should be included in the shipment to enable the laboratory workers who receive the shipment to expedite appropriate tests.

It is advisable that shipments be made by air express. The laboratory receiving the shipment should be notified of the name of the airline carrying the package, the flight number, and the time of arrival at the airport.

VI. Prophylaxis and control:

Between 1900 and 1940, numerous attempts were made to develop vaccines for the prevention of rinderpest. Trial vaccination of cattle with suspensions of inactivated lymphatic tissues and whole blood were largely ineffective. Historically, it is of interest to note that Robert Koch, during the cholera epidemic in Egypt in 1897, inoculated goats with infectious cattle blood and observed only pyrexia after an incubation of 2-3 days. The virus was passaged in this species 7 times, and returned to cattle after 2 and 5 goat passages. Dr. Koch considered that slow attenuation would probably be possible by repeated passages in goats. In 1930, Edwards, in India, reported the development of the first attenuated virus vaccine by continual passage of the virus in goats. In 1940, Nakamura, working in Korea, improved the vaccine by passaging the virus in rabbits.

Although the goat vaccine left much to be desired, it provided--for the first time--a means to vaccinate cattle and buffaloes against the disease. The later "lapinized" or rabbit-passaged virus was the first acceptable vaccine, and its use helped to turn the tide of the increasing frequency of outbreaks in enzootic areas. The cost of preparing the vaccine was considered prohibitive, particularly for use in developing countries, and also, it could not be supplied in sufficient quantities for large-scale vaccination. Moreover, approximately 2% of the animals vaccinated contracted the disease and were destroyed.

The adaptation of rinderpest virus to propagation in tissue cultures prepared from calf kidneys in the late 1950's, and the development of an attenuated live virus vaccine shortly thereafter, opened the way for mass vaccination in all parts of the world. The adaptation of the virus to tissue culture made possible the development of a rapid diagnostic laboratory test. Where the disease has been found to be prevalent, it is of particular importance that the new tissue culture vaccine can now be prepared rapidly, at a cost acceptable for use in developing countries. During the past 8 years, a massive campaign to eradicate rinderpest by mass vaccination of cattle and water buffaloes has been in progress in the old, as well as the developing countries of Africa, with the assistance of FAO, AID, and the native governments. While the mass vaccination, revaccination, and followup program has been carried out systematically in many African countries with gratifying results, the same cannot be said of a number of countries in the Middle East from Lebanon to the Persian Gulf where renewed outbreaks have occurred during the past months.

Control: Prohibition of importation of susceptible animals and animal products from countries where the disease exists. Hold animals destined for zoological gardens in quarantine until they are known to be free of rinderpest. If they are infected, the animals should be slaughtered and incinerated. Control over movements of cattle and other susceptible animals in enzootic areas of the world should be exercised by the authorities.

With the laboratory procedures for rapid identification of the disease available, and the ability to produce large quantities of tissue culture vaccine, there is no valid reason to permit the continued existance and spread of the dreaded disease in any part of the world. Cooperative efforts among various international organizations such as the Food and Agriculture Organization of the United Nations, the Agency for International Development, various philanthropic organizations, and the native governments involved, have demonstrated the ability to mount an effective campaign to eradicate the disease by systematic means and follow-up examination of all susceptible animals.

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SHEEP POX*

I. Identification of disease:

A. Definition:--Sheep pox (variola ovina, Clavelée-Pockenseuche) is a highly contagious viral disease of sheep characterized by erythematous eruptions on the skin. Early in the course of the disease, sheep pox lesions are papular but later progress to pustular eruptions. When the lesions are generalized, they may be associated with hemorrhagic inflamation of the respiratory and gastrointestinal mucosae and high mortality.

B. Etiology.--The viral entity of sheep pox and its characteristic inclusion bodies were established in 1902. The size of sheep pox virus is 200-250 x 150-200 mu. It has been propagated in a variety of cell cultures, but it replicates best in lamb kidney and testicle cultures, producing cytopathogenic effects and cytoplasmic inclusion bodies. Passage of the virus in cell cultures or chicken embryos results in attentuation for sheep. Although all virus strains of sheep pox are serologically identical, strains may vary in virulence.

Under natural conditions sheep and goat pox viruses are host specific. However, their immunogenic relationship was confirmed by reciprocal cross protection, complement fixation (CF) and agar gel diffusion precipitin (AGDP) tests.

Sheep pox virus is related antigenically to Neethling lumpy skin disease virus. Although cross protection was not demonstrated between sheep pox and contagious pustular dermatitis, its serological relationship was shown by the CF and AGDP tests.

Sheep pox virus in lymph retains its infectivity for 2-3 years at -15°C and for 2-3 weeks at 25-37°C. It is inactivated when heated

*Prepared by A.H. Dardiri

3 hours at 50°C, 15 minutes at 52-53°C and 3 minutes at 56-58°C. Virus produced in cell culture is inactivated by ether and chloroform after 18-24 hours exposure. Sheep pox virus in lymph is inactivated in 5 minutes by 2% phenol, 0.5-1% formalin, 0.1-1% hypochloride, 2% hydrochloric acid, sulphuric acid and 1:10,000 tincture of iodine.

C. History.--Sheep pox has been described in Europe since the first century A.D. It was reported in England, France, Italy and Germany during the 13th century. Severe epizootics were encountered in the countries of Southeastern Europe and the Mediterranean regions, approximately 1,000,000 sheep succumbing to the disease in 1805. Through the use of vaccine and application of sanitary measures, the disease was controlled in Britain, Germany and other Western European countries before the end of the 19th century. Foci of infection still exist in France, Spain and Portugal.

II. Signs:

A. Clinical Features.--The initial disease signs are fever, lacrimation, salivation and serous nasal discharge. Approximately 2 days later eruptions develop in the sparsely wooled areas of the skin such as the groin, scrotum, area below the tail, eyelids, lips, cheeks, nostrils, udder and vulvar labia. Sheep pox lesions begin as macules with a slight edema of the surrounding skin. Later the lesions develop into papules followed by pustules. The formation of pustules may be preceded by the development of vesicles; however, development of vesicles is not always observed. As the surfaces of the pustules dry out, thin scabs are formed. The benign form of the disease is more common in adult animals with skin lesions, particularly under the tail, and a mild systemic reaction and mortality of about 5-10%. A malignant form of sheep pox is more common in lambs, with depression, generalized and coalescent skin

lesions and frequently other lesions in the buccal, digestive and respiratory mucosae. Secondary bacterial infection may elicit a second temperature rise. Mortality can reach 80% of the effected flock in this severe form.

Susceptibility to sheep pox varies with age, breed, resistance of individual animals, and husbandry of the flock. The course of the disease is usually 3-4 weeks. Certain breeds of sheep are resistant to natural and experimental infection. Marino sheep are highly susceptible.

B. Incubation period.--The incubation period of sheep pox varies from 2-14 days. Usually it is shorter during hot weather.

III. Pathologic changes:

A. Postmortem lesions.--Skin eruptions or pocks on the areas of the skin devoid of wool are common. The cutaneous areas surrounding these lesions are hyperemic with edema of varying degree. All, or a combination of lesions, such as papules, vesicles, pustules, pocks and scabs may be found. Rupture of the pustules usually results in matting of the wool surrounding the pustule. Lesions in lambs are often coalescent. In the malignant form, pox lesions may extend into the mucosa of the mouth, pharynx, larynx and vagina. Small grayish lymphoma-like or caseated nodules surrounded by pneumonic areas are often found in the lungs and kidneys.

B. Microlesions.--Edema of the dermis with infiltration of polymorphoneculear cells is noted in the macular stage of sheep pox. In the papular stage, large cells resembling histiocytes are present. Later, these cells are transformed into "sheep pox cells" or "cellules claveleuses", which are large cells with oval or irregular nuclei. Some of these cells contain granular cytoplasmic inclusion bodies. Accompanying

the appearance of "pox cells", is an inflammatory edema which may extend to the subcutis. Necrosis and thrombosis of the dermal veins are often present. Scab portions of the lesion consist of exudate and a pellicle of necrotic epidermis.

IV. Diagnosis:

A. In the field.--Appearance of a progressive pox-like disease in a susceptible sheep flock is suggestive of sheep pox, especially when asssociated with movement of animals or introduction of new stock. However, clinical diagnosis may be difficult in the mild form where lesions are confined to small areas of the skin.

B. Laboratory.--Direct light microscope examination of stained smears from fresh lesions will assist in the diagnosis of sheep pox by revealing typical inclusion bodies. Electron microscope examination of lesions material will rapidly demonstrate morphology of the virus particle. Identification of sheep pox antigen in infected cells by staining with fluorescein or ferritin conjugated antibodies is necessary for a confirmatory diagnosis. The virus may be isolated from the blood, lymph and lesions of affected animals during the viremic stage and by inoculation of cell culture, chicken embryos or susceptible sheep. Detection of specific antibodies in the serum of recovered animals can be done by virus neutralization, CF, AGDP and fluorescent antibody (FA) tests. Complement fixation antibodies are detected in the serums of vaccinated or artifically infected sheep as early as the 7th day after inoculation and reach a peak at 21-26 days postinoculation (DPI). Precipitating antibodies are detected as early as 14 DPI. The following specimens may be submitted to the laboratory frozen with dry ice: 1) blood from sheep during the febrile disease stage, 2) lymph node and scab lesions, and 3) serum obtained at the acute and

convalescent stages of the disease. A portion from skin lesions should be prepared in buffered glycerin.

C. Differential Diagnosis.--Formation of scab-like lesions are common to sheep pox, eczema and scabies. Eczema is non-infectious whereas the last is a parasitic disease. In their non-complicated form none of them are associated with a febrile reaction. The mouth lesions and the systemic reaction of sheep pox may be confused with those of peste des petits ruminants (PPR). Lack of papule and pustule formations on the skin and presence of necrotic ulcerative stomatitis in animals infected with PPR will assist in the differential diagnosis of the two diseases. Sheep pox may also be confused with contagious ecthyma (CE); however, proliferative lesions of the disease around the mouth in and

V. Prognosis:

Mortaility from sheep pox varies from 5-80%. Losses from the disease may be increased by the stress factors of severe hot weather and unsatisfactory snaitary husbandry practices. Outbreaks of sheep pox are more common during the summer. Lesions as well as mortality are more severe in lambs than in older sheep. The peak of mortality is usually within 2 weeks following appearance of generalized and coalescent lesions. Severity of the disease varies with each epizootic and the particular breed of sheep involved.

VI. Epizootiology:

A. Geographical Distribution.--Sheep pox in various areas of Europe, Asia and Africa and is enzootic in Iran, India and neighboring countries. Foci of the disease are found in Spain, Portugal and Russia. It has been reported in Egypt, the Sudan, Ethiopia and Kenya. It has not

been recognized in North and South America.

B. Transmission.--Outbreaks originate by introduction of or contact with infected sheep. Sheep pox is spread by aerosols from nasal secretions, saliva and dried scabs. The disease is transmitted by direct contact of susceptible and sick animals and indirectly by contaminated fomites and transport vehicles. Experimental disease transmission is possible by intradermal, subcutaneous, intravenous, intraperitoneal and intracerebral inoculation. Administration of the virus into the nasal cavity or trachea provokes generalized disease in susceptible sheep. Sheep pox virus may remain viable in wool for 2 months and on contaminated premises for as long as 6 months.

C. Hosts.--Sheep are the natural hosts for sheep pox virus. The virus is host-specific although infection of other species can occur experimentally. Artificial exposure to sheep pox protects cattle against lumpy skin disease. Some strains of sheep pox virus can be adapted to goats with loss of virulence for sheep.

VII. Control and Eradication:

A. Preventive Measures.--The introduction of sheep from countries where sheep pos is enzootic to those countries free of the disease should be prohibited.

B. Control and Eradication.--In the United States of America the disease must be reported to the State and Federal regulatory agencies. Imposition of quarantines on infected premises is mandatory. Movement of sick and contact sheep should be prohibited. Laboratory diagnosis should be obtained as soon as possible. Infected and contact sheep flocks should be slaughtered and the carcasses disposed of properly. Infected premises should be thoroughly cleaned and decontaminated.

C. Immunization.--Two types of live, virulent vaccines were commonly used until the pox virus was attenuated by passage in both chicken embryo and lamb kidney cell culture. Mild virus strains which did not cause generalized lesions following subcutaneous or intradermal inoculations were used in Iran and Egypt. Later in Russia an attenuated strain of sheep pox virus was obtained by passage through chicken embryo cultures. Its safety and efficiency as an immunizing agent was proven by extensive field trials. More recently, sheep pox virus was attenuated after 30 serial passages in sheep kidney cell cultures and used in Iran. The duration of immunity obtained with this vaccine under laboratory and field conditions was at least 22 months, combination of this vaccine with one against anthrax administered as a single injection resulted in a solid immunity against both diseases. Trials conducted in India to develop chemically inactivated vaccines. Crude virulent virus was inactivated after 48 hours with 0.01% formalin. This product adsorbed to aluminum hydroxide gel, protected sheep for at least 4 months.

VIII. Public Health:

Man is not susceptible to sheep pox under natural conditions. Sheep slaughtered during the febrile of pyemic stages of sheep pox are not suitable for human consumption.

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SWINE VESICULAR DISEASE *

I. Identification of disease:

A. Definition.--Swine vesicular disease (SVD) is a contagious, viral infection of swine. It is characterized by vesicular lesions and subsequently by erosions of the epithelium of the mouth, nares, snout, and feet.

B. Other vesicular diseases.-- The clinical lesions and signs of pigs suffering from SVD are indistinguishable from those due to foot-and-mouth disease (FMD), vesicular stomatitis (VS), and vesicular exanthema of swine (VES).

C. Etiology.--Swine vesicular disease is caused by an enterovirus of the picornaviruses (1). It is not affected by acid, pH or ether, contains single-stranded RNA and is of 280 Å diameter. Its density in cesium chloride is 1.34 grams per ml and it has a sedimentation rate of 150 S. It is stabilized by 1M $MgCl_2$ at 50°C.

The virus of SVD is closely related to Coxsackie B-5 which is a human enterovirus (5). All strains of SVDV are neutralized by anti-Coxsackie B-5 virus antiserums and serum from pigs recovered from SVD will neutralize Coxsackie B-5 virus in tests in tissue cultures of mice. Antiserums to other pig enteroviruses show no neutralizing activity against SVDV. Pig serums collected in the field from animals in the U.S. did not neutralize SVDV whereas several human serums did, indicating that an infection had occurred in these persons by a virus related to SVDV.

Strain variation among different isolates of SVDV can be readily demonstrated by double diffusion reactions in agar (6). By use of this

*Prepared by J.J. Callis, J.H. Graves and P.D. McKercher

procedure it was shown that illness that had occurred in some laboratory workers was due to infection by SVDV and not Coxsackie B-5 virus. Hybridization studies of the RNAs from different SVDV strains and Coxsackie B-5 show about 50% homology between SVDV and the Faulkner strain of Coxsackie B-5 (Brown, personal communication). However, it also has been shown that Coxsackie B-5 virus recently isolated from human cases shown about the same degree of identity with the B-5 strains isolated 20 years ago, indicating a variation of the virus has occurred through the years.

Based on the absence of evidence of any previous infection of pigs with a virus serologically related to SVDV and its close relationship to the human virus, Coxsackie B-5, it has been suggested that SVD is a relatively new disease of pigs caused by a virus of human origin (5).

D. History:-- Swine vesicular disease was first recognized as a distinct entity of swine in Italy in 1966, where Nardelli et al. (1) reported on a syndrome in pigs that resembled foot-and-mouth disease (FMD). The disease was milder but the lesions were indistinguishable not only from those of FMD, but vesicular exanthema of swine (VES) and vesicular stomatitis (VS). Nardelli, together with British colleagues, indicated that the agent responsible for this outbreak was a porcine enterovirus. Following the initial outbreak the disease subsided and the peculiar incident was soon forgotten.

In 1970, Mowat et al. (2) from the Animal Virus Research Institute at Pirbright, England, noticed a vesicular condition in pigs in an area of Hong Kong where they were conducting trials with experimental FMD vaccines. The disease differed from FMD only in that the morbidity was lower, lesions less severe and there was no mortality. If first appeared in pigs on a farm where the swine had not been vaccinated with the experimental vaccine.

It subsequently spread to neighboring farms and others up to seven miles away. Repeated tests failed to show the virus to be any of the seven serotypes of FMDV. Bearing in mind that one of the seven recognized types of FMDV was first identified in material sent from Asia, the British workers were aware that this outbreak might be due to a new serotype. The possibility that a new serotype may evolve in the field must always be kept in mind, and especially with virus from this area where movement of animals is frequent with China to the north. The problem was simplified by the previously recorded Italian outbreak and the availability of diagnostic materials. The results of the tests indicated a close similarity between the viruses from the Hong Kong and Italian outbreaks which led to Hong Kong virus being classified as a porcine enterovirus.

In December of 1972 (3), a vesicular disease occurred among pigs on a farm in England and based on clinical signs was confirmed as FMD. Five days later, however, when repeated serological tests failed to identify FMD antigen, the identification of the agent as an enterovirus was confirmed. By comparison with the Hong Kong and Italian viruses, it was concluded that the vesicular disease occurring in England was due to a virus similar to that which had been identified in Italy and Hong Kong. It was evident that a new disease entity was present in England. The eradication procedures that had been already instituted were modified in that only swine were slaughtered.

The European Commission for the Control of Foot-and-Mouth Disease, FAO convened a meeting in Rome in January of 1973, to assess the situation in Europe and to adopt standard diagnostic procedures (4). At this meeting it was disclosed that SVD had continued to occur periodically in

Italy since 1966, but that the lesions were milder than those originally observed. Further, the delegates from Poland and Austria advised that SVD had also occurred recently in their countries. To date, the disease in Europe has also been diagnosed in France, Switzerland, West Germany, and Belgium, and in Asia, in Taiwan and Japan. Efforts to eradicate the disease in England have continued and according to the latest reports it has occurred on a total of 259 premises and the British Government has spent 11 million dollars in an effort to eradicate it from that country. The disease status in at least some of the other countries is not known and it may be endemic in some. In some countries, for example Belgium and Japan, it has been eradicated by slaughter of infected and exposed animals and decontamination of the premises.

Swine vesicular disease is of great economic importance. It causes vesicles on the feet, snout and in the mouth resulting in lameness and difficulty in eating. The animal loses weight and becomes unthrifty. The economic importance of the disease to a country that exports swine or pork products is greater because countries free from the infection usually close their markets to products from the affected country. The direct costs of eradication may also be considerable.

II. Signs:

A. Clinical features.--The clinical signs and lesions of SVD are indistinguishable from those of FMD, VS, and VES (see foot-and-mouth disease). The first evidence of infection is lameness which often precedes the appearance of vesicles by several hours. Diseased pigs have fever of 104 to 106 F or greater. Feed comsumption is reduced, probably due to reluctance to move to the feed troughs.

The lesions are vesicular and appear on the coronary bands, soles of the feet, interdigital spaces and tongue, nares, and lips. Ulceration of the skin over the metacarpal and metatarsal regions is common. While not documented, abortion or death of newborn pigs has been observed. The vesicles rupture and often epithelial areas of feet not originally vesicular will lift away and form raw ulcerous areas. Occasionally, the hoof will be shed from the infected foot.

There is increasing evidence that a subclinical form of SVD occurs or a mild form that goes undetected unless careful examination is made. This has complicated eradication campaigns in that the disease can be widely disseminated before the diagnosis is made.

Preliminary studies of SVD at PIADC indicated that infected pigs developed encephalomyelitis (9). In Italy, Gagliardi et al. (10) have seen encephalitis as a clinical feature of the disease in at least three different breeding establishments. Some signs of central nervous system involvement consisted of a very unsteady gait resulting in short stiff-legged steps as if unsure of their equilibrium. Shivering occurred across the flanks and chorea type leg movements were frequently observed (Graves et al., unpublished data). In one experiment, a high mortality rate was found in newborn pigs. In this experiment a sow was infected by intravenous inoculation of SVDV-UKG within 15 hours after delivering 9 normal pigs. All of the piglets were dead by the third day after the appearance of clinical signs in the sow (McKercher et al., unpublished data).

Swine apparently are the only species of livestock susceptible to SVD.

B. Incubation period.--Susceptible animals in contact with SVD-infected pigs will show signs of the disease in 2 to 7 days. If the virus is inoculated, signs may be seen as soon as 30 hours after inoculation. Pigs fed contaminated feed showed evidence of SVD in 2 to 3 days.

III. Pathologic Changes:

A. Pathogenesis.-- The primary sites of infection in SVD appear to be the intestinal tract. Infection is readily obtained by feeding the virus. The disease can be induced by inoculating the virus on the feet or intravenously. The sensitivity of other routes of infection has not been extensively studied.

Virus is widely disseminated in the infected animal being found in essentially all tissues. It is readily isolated from the feces, blood, and OP samples of diseased pigs.

Neutralizing antibody against SVDV can be found in the serum of infected pigs by the 7th day postinfection rising to a peak by the 28th day (11). Neutralizing antibody persists in recovered pigs for at least 60 days after infection at which time the recovered swine are refractive to infections (McKercher and Graves, unpublished date). An experimental vaccine was prepared by inactivating SVDV grown in IBRS-2 cells for 72 hours with 0.05% acetylethyleneimine at 25°C and combining the inactivated antigen with an oil adjuvant. Vaccinated pigs were refractive to challenge by inoculation or exposure to infected animals at 60 days postvaccination (McKercher, 1973, unpublished date).

B. Post-mortem lesions,--Extensive study of the pathology of SVD-infected pigs has not been reported. Evidence of damage to the feet

from vesiculation may be seen in pigs up to 30 days after recovery. Monlux et al. (1974) found extensive microscopic lesions including perivascular cuffing and infiltration by polymorphonuclear leucocytes, other lesions were related to vesiculation and similar to FMD.

IV. Diagnosis:

A. Field diagnosis.--Any vesicular disease in pigs is of major importance. It is impossible to distinguish SVD from the other vesicular diseases of swine in the field, but information on the possible involvement of cattle in the area may be an important clue. A word of caution, however, is that occasionally a strain of FMD may be present that does not readily infect cattle.[1] (See the section on foot-and-mouth disease for differential diagnosis of vesicular diseases.)

B. Field samples.-- See chapter on FMD and section on collection of samples for vesicular diseases.

C. Laboratory diagnostic tests.--(See chapter on FMD). Differentiation from FMD, VES and VS can only be done by use of laboratory serological procedures. Of these the complement-fixation (CF) test is more rapid than the serum neutralization test.

Antiserums for use in the CF test are prepared by immunizing guinea pigs with SVDV antigen purified from tissue cultures or virus recovered from the brains of infected baby mice. The test antigen used for the differential diagnosis usually consists of a suspension of vesicular lesion material collected from the diseased animal.

Differential diagnosis by serum neutralization can be done by mixture of the test virus with the guinea pig serums used in the CF test and then

inoculating this mixture into tissue culture cells susceptible to virus infection.

Differences among strains of SVDV can be demonstrated by observation of "spurs" that occur where heterologous and homologous antiserums are used in double diffusion precipitin reactions in agar (6). Such differences are of importance in epizootiological studies of SVD and are not of concern in the differential diagnosis within the vesicular disease group.

Some differentiation can be shown by inoculating the test virus into different lines of tissue culture cells (IBRS, PK 15). The SVDV had only been shown to grow in cells of pig origin whereas FMDV will grow in a variety of bovine cells and BHK cell lines.

Newborn mice less than 24 hours old are susceptible to SVDV and will die within 3 to 7 days post intra-cranial inoculation. Mice become resistant when older than 6 days.

V. Prognosis:

Prognosis of recovery from SVD is generally favorable; however, the existence of this disease as an endemic problem will severely hinder the diagnosis and ready detection of the presence of FMD.

VI. Epidemiology:

A. Geographical distribution.--Currently (1975), SVD has been confirmed as occurring in Italy, Poland, Austria, France, Great Britain, Hong Kong and Japan.

B. Transmission.--The primary mode of transmission is by contact of susceptible pigs with the excretions of infected pigs. The virus is much more resistant to disinfectants and environmental conditions than

FMD virus and during the attempted eradication of the disease from Great Britain in 1973, infection of susceptible animals used to restock decontaminated premises proved a problem. During this same campaign it was found that trucks that had carried infected pigs and had been decontaminated by standard FMD procedures were a major source of subsequent spread of the disease.

Investigations of outbreaks of SVD seem to incriminate the feeding of garbage contaminated with SVDV-infected meat scraps. The stability of the virus is such that it is not inactivated by the acid changes that occur in the musculature after death; thus, the virus can be expected to withstand the various processes used in the production of sausages such as salami. Dried salami and pepperoni sausages prepared from the meat of infected swine were found to contain residual SVDV for at least 200 days. Processed intestinal casings from infected swine, packed in salt and stored at 39 F, contained virulent virus for a similar period. Thus, imported pork products produced in countries where SVD is present are potential sources of infection to a susceptible swine population (16, 17).

Those cured and dried products originating from countries where SVD is present are not permitted entry into the United States except for further processing by heating to an internal temperature of 166 F.

C. Hosts:--The only known species susceptible to infection with SVD are swine, baby mice, and man. (See Table 1).

VII. Control and Eradication:

A. Prevention.--(See foot-and-mouth disease.)

B. Control and eradication.--(See foot-and-mouth disease.)

C. Treatment.--(See foot-and-mouth disease.)

D. Immunization.--An experimental vaccine against SVD has been reported (12, 13, 14). Although vaccines have not been used other than experimentally, the data provided would indicate that they are quite adequate.

E. Import restrictions.--(See foot-and-mouth disease.)

VIII. Public Health Aspects:

Swine vesicular disease virus is closely related to the human enterovirus, Coxsackie B-5. Human infection has been reported in laboratory workers and most human serums will show some neutralization of SVDV. Caution should be taken in handling highly virus-contaminated materials and unnecessary human contact with diseased pigs should be avoided.

TABLE 1.

SUSCEPTIBILITY RANGE TO VIRUSES OF FOOT-AND-MOUTH DISEASE (FMD), VESICULAR EXANTHEMA OF SWINE (VES), VESICULAR STOMATITIS (VS), AND SWINE VESICULAR DISEASE (SVD)

Species	Virus			
	FMD	VES	VS	SVD
Horse	0	±	+	0
Bovine	+	0	+	0
Swine	+	+	+	+
Sheep & Goats	+	0	+	0
Man	± (Rare)	0	+	±
Mice, 1-2 days	+	0	+	+
Adult Mice	±	0	+	0

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VESICULAR EXANTHEMA OF SWINE *

I. Identification of disease:

A. Definition.--Vesicular exanthema of swine (VES) is an acute, febrile, contagious viral disease of swine, characterized by formation of vesicles on certain parts of the body.

B. Etiology.--The causal agent of VES is a virus of the picornovirus group, and it is different from the other three major vesicular diseases, foot-and-mouth disease (FMD), vesicular stomatitis (VS), and swine vesicular disease (SVD).

C. History.--On April 22, 1932, a vesicular disease affecting swine only was first reported from a hog ranch near Buena Park, Orange County, California. The 1932 outbreak was diagnosed FMD and animals involved were slaughtered. In 1933 a similar disease occured in San Diego, about 100 miles distance from the 1932 focus. Following this outbreak, the malady was correctly described as a new disease. Sporadic outbreaks occurred in the USA until 1956, the last taking place in New Jersey that year.

II. Signs:

A. Clinical features.--A characteristic temperature rise is usually followed by vesicles of the snout, lips, or oral cavity as well as the coronary band and interdigital regions of the feet.

B. Incubation period.--Primary lesions develop within 12-48 hours and secondary lesions 12-48 hours later.

III. Pathological changes:

A. Post mortem.--Vesicles and similar lesions affecting the dermis and contiguous mucosae on the affected parts constitute the usual lesions.

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*Prepared by R. J. Yedloutschnig and F.W. Wilder

B. Microlesions.--Virus replicates in the Malpighian layer of the epidermis; concurrently, the stratified squamous epithelial cells undergo marked swelling of their cytoplasms. Following this the cells become necrotic; their deterioration spreads virus to non-infected cells continuing the formation of lesions.

IV. Diagnosis:

A. In the field.--Pyrexia, vesiculation (and related lesions), and lameness are always present. It should be remembered that actual vesicles may rarely be seen as they are frequently ruptured. The vesicles and lesions are not distinguishable from those of other vesicular diseases.

B. Laboratory.--Laboratories utilize various tests, including complement fixation, virus neutralization, isolation, and identification of the virus. Animal inoculation is frequently employed in the laboratory and sometimes in the field.

C. Differential diagnosis.--Final confirmation of the diagnosis can only be made by the laboratory tests mentioned above. However, the inoculation of equine, bovine, and porcine species brought from an adequate distance from the outbreak, may be used to reach a presumptive diagnosis. Equine, bovine, and porcine species are affected by VS; bovine and porcine are both affected by most strains of FMDV; VES affects only swine. The USA is now free of VES; however, since VES has been regarded as an "extinct" disease, any new vesicular outbreak affecting only swine would require laboratory determination as to whether it was a recrudescence of VES or a first outbreak of SVD.

V. Prognosis:

A severe weight loss may occur during the course of the disease but recovered of uncomplicated cases is usually prompt and without sequelae.

Fatal secondary infections may occur.

VI. Epizootiology:

A. Geographic distribution.--The disease has only been diagnosed in continental USA, except for a single appearance in Iceland in 1955 which was caused by feeding local swine garbage containing porcine scraps from the USA.

B. Transmission.--The disease is spread by direct contact and by feeding raw garbage. The link between raw garbage and the disease is apparently the retention of infective virus in pork scraps. Recent findings indicate that the Sea Lion may have played a part in transmission.

C. Hosts.--The only known host is swine. The role of the Sea Lion is being studied; a virus similar to VESV recovered from Sea Lions has produced experimental vesicular lesions in swine.

VII. Control and eradication:

A. Preventive measures.--Slaughter and quarantine of infected and exposed swine and decontamination of premises are the most effective measures. There must be absolutely no feeding of raw or improperly cooked garbage to swine.

B. Sanitation and disinfection.--A solution of 2% sodium hydroxide is a practical disinfectant. A viricide having a high pH is required to denature VES viral protein.

C. Treatment.--None.

D. Immunization.--There is no vaccine available. At the present time there are about a dozen distinct antigenic types of VESV known. Since there have been no outbreaks since 1956, research on this subject has been extremely limited.

VIII. Public health aspects:

The virus does not infect man.

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O U T L I N E G U I D E

Collection and Submission of Specimens

in Suspected Foreign Animal Diseases *

For Demonstrations and Exercises

in Foreign Animal Disease Courses **

PLUM ISLAND ANIMAL DISEASE CENTER

* Prepared by F.W. Wilder, J. Kopec and A. H. Dardiri

** See also DIAGNOSIS OF SUSPECTED EMERGENCY ANIMAL DISEASES (Part IIIB in "Emergency Animal Disease Eradication Guide", pages 57-67. Appendix 2)

Introduction

The capability of a laboratory to confirm the diagnosis of a suspected exotic animal disease is directly related to the types, amounts, and conditions of the specimens submitted. Therefore, it is important that each field diagnostician become intimately familiar with this subject.

This outline is organized in approximately the same order as you would perform your specimen collection in the field. Keep in mind that specimens for agent isolation should be collected as aseptically as possible; these should always be collected prior to those for histopathology which need only be clear and fixed in 10% neutral buffered formalin.

Only diseases demonstrated in the PIADC foreign animal disease courses are included in this outline. The technics outlined are very similar, but in less detail, than those given in the well-known "Red Book" (Emergency Disease Eradication Guide). The entire part III B of this book, covering the collection and submission of specimens is included as Appendix 2, for your convenience. It should be remembered that the outlines have been developed to emphasize the principles of proper collection of laboratory specimens. Some variations in technic are included. For example, some laboratory personnel prefer tissues for histopathology cut 3 mm instead of one-fourth inch (approximately 6 mm) in thickness and fixed in 50 X rather than 10X the specimen volume of fixative.

Specimens

Certain basics such as the following should be kept in mind:

1. Ordinarily, put each tissue in a separate container, correctly labelled.
2. Use some method of labelling which cannot be lost or destroyed easily. For instance, adhesive tape should go entirely around

the vial or bottle and overlap so that moisture does not dislodge it.

3. Writing should be with pencil or ink which will not smudge or blur when wet.
4. Vials, tubes, or bottles to be frozen should be no more than half full. These should be sturdy enough to withstand freezing and dropping.
5. A history should accompany specimens.

SUGGESTIONS FOR IMPROVEMENT OF COLLECTION AND SUBMISSION METHODS:

You are invited to turn in to the director any suggestions for improvement. (Remember, that the APHIS "Red Book" is the authority for field diagnosticians in the collection and transmission of specimens.)

AFRICAN HORSE SICKNESS

1. Blood.
   a. Whole blood, in an equal volume of oxalate, phenol (carbolic acid), and glycerin (OPG or OCG) solution. Collect at height of pyrexia. Refrigerate only. (For virus isolation.)
   b. Serum, 20 ml, from acutely ill and convalescent animals, frozen. (For serology.)
   c. Blood smears; at least 6 slides, air dried and fixed in absolute methyl alcohol. (For differential counts.)
2. Tissues, collected aseptically, frozen. (For virus isolation.)
   a. Spleen, in 50% buffered glycerin.
   b. Liver.
   c. Lung.
   d. Lymph nodes draining affected regions (thoracic, mediastinal, and mesenteric).
3. Tissues, not over ½" thick, fixed in 10X tissue volumes of 10% neutral buffered formalin. (For histopathology.)
   a. Spleen.
   b. Liver.
   c. Lung.
   d. Kidney.
   e. Heart.
   f. Lymph nodes.
4. Formulas. See appendix 1.
5. History to be submitted with specimens. See appendices 3 and 5.

AFRICAN SWINE FEVER (AND HOG CHOLERA)

1. Blood.
   a. Whole blood, heparinized, frozen. (For virus isolation.)
   b. Serum, 50 ml, frozen. (For serology.)
2. Tissues, collected aseptically, frozen. (For virus isolation and FA tests.)
   a. Spleen.
   b. Lymph nodes (cervical and visceral).
   c. Liver.
   d. Kidney.
   e. Tonsil (whole or biopsy).
   f. Ileum (terminal 3 inches). NOTE: SEE APPENDICES 2 AND 6.
3. Tissues, ¼" inch or less thick, fixed in at least 10X their volume of neutral 10% formalin.* (For histopathology.)
   a. Spleen.
   b. Tonsil.
   c. Liver.
   d. Adrenal.
   e. Kidney.
   f. Lymph nodes (body and visceral).
   g. Brain (fixed intact). NOTE: SEE APPENDIX 2.
4. Formulas. See Appendix 1.
5. History to be submitted with specimens. See appendices 3, 5, and 6.

*Some laboratory personnel recommend that tissues for histopathology be cut 3 mm thick and that all tissues for histopathology be fixed in as much as 50X the tissue volume of fixative.

DERMOPATHIC BOVINE HERPESVIRUS INFECTIONS AND LUMPY SKIN DISEASE

1. Blood.
   a. Whole blood, frozen. (For virus isolation.)
   b. Serum, 20 ml from acutely ill and convalescent animals and paired sera from an individual animal in acute and convalescent phases 92 to 3 weeks after acute stage). (For serology.)
2. Tissues, frozen. (For virus isolation.)
   a. Skin lesions. Biopsies should be taken of at least 2 lesions. Scrapings from tissues and scabs, vesicular contents. (Examine muzzle, teats, mammary skin carefully.)
   b. Lymph nodes, swollen.
3. Tissues, not over ¼" thick, fixed in 10X tissue volume of 10% neutral buffered formalin. (For histopathology.)
   a. Skin lesions (biopsies).
   b. Lymph nodes, swollen.
4. Formulas. See appendix 1.
5. History to be submitted with specimens. See appendices 3 and 5.

CONTAGIOUS BOVINE PLEUROPNEUMONIA

1. Blood.
   a. Whole blood, 20 ml, refrigerated or frozen. (For agent isolation.)
   b. Serum, 20 ml, refrigerated from acutely ill and convalescent and paired sera from individual animals in the acute stage and 2-3 weeks later.
2. Body fluids. Collect aseptically, refrigerate or freeze. (For agent isolation.)
   a. Pleural fluid, 10 ml or more.
   b. Lung lesion exudate.
3. Tissues collected aseptically, frozen or refrigerated. (For agent isolation.)
   a. Lung lesions.
   b. Lymph nodes (bronchial and peripheral, when enlarged).
   c. Spleen, when engorged.
   d. Thyroid glands.
4. Tissues, not over ¼" thick, fixed in 10X tissue volume of neutral buffered 10% formalin.
   a. Spleen.
   b. Liver.
   c. Lung.
   d. Kidney.
   e. Brain, including floor of 4th ventricle.* (See appendix 1b.)
   f. Intact eye and conjunctiva.*
5. Formulas. See appendix 1.
6. History to be submitted with specimens. See appendices 3 and 5.

*For use in differentiating malignant catarrhal fever.

FOWL PLAGUE (AND NEWCASTLE DISEASE)

1. Whole birds. Submit whole birds if practical; as many as 3 acutely ill and 3 dead birds are requested by the laboratory. If this is not feasible, send the following specimens from several birds:
2. Blood.
   a. Whole blood, heparinized, frozen. (For virus isolation.)
   b. Serum, frozen. (For serology.)
3. Tissues, collected aseptically, frozen. (For virus isolation.)
   a. Spleen.
   b. Trachea.
   c. Lung.
   d. Proventriculus.
   e. Brain.
   f. Small intestine.
   g. Liver.
   h. Kidneys.
   i. Bone marrow.
   j. Cecal tonsils.
4. Tissues, cut less than ¼" thick and fixed in 10X their volumes of 10% neutral buffered formalin. (For histopathology.)
   a. Brain.
   b. Spleen.
   c. Trachea.
   d. Lung.
   e. Proventriculus.

f. Ventriculus (gizzard).

g. Small intestine.

h. Liver.

5. Formulas. See appendix 1.

6. History to be submitted with specimens. See appendices 4 and 5.

LUMPY SKIN DISEASE: See Dermopathic Bovine Herpesvirus Infections.

NEWCASTLE DISEASE: See Fowl Plague.

PESTE DES PETITS RUMINANTS: See Rinderpest.

FOOT-AND-MOUTH DISEASE (AND OTHER VESICULAR DISEASES INCLUDING VESICULAR STOMATITIS, VESICULAR EXANTHEMA OF SWINE, AND SWINE VESICULAR DISEASE)

1. Vesicular specimens.
   a. Vesicular fluid, if available. Collect from unruptured vesicle separately; freeze.
   b. Vesicular lesion tissue. Collect about 5 grams in phosphate buffered glycerin. (Volumetric measurement of 5 cc liquid may serve as guide.)
2. Probang specimens (ruminants only). "Probang (O-P) fluids should be submitted in all cases, even though accompanied by voluminous vesicle harvest from the same animal." Director, Emergency Programs, change in "Red Book" 15 Nov. 1972.
   a. Esophageal-pharyngeal (O-P) fluid; 10 ml in equal amount of phosphate buffered glycerine.
3. Blood.
   a. Whole blood, 10 ml collected during febrile period, frozen. (For virus isolation.)
   b. Serum, 10 ml, from animals in the acute and convalescent stages. (For serology.)
4. Fecal sample. (For swine vesicular disease.) Collect from animals with and without lesions and contact animals. Freeze. (For virus isolation.)
5. Formulas. See appendix 1.
6. History to be submitted with specimens. See appendices 3 and 5.

MALIGNANT CATARRHAL FEVER (AFRICAN)

1. Blood.
   a. Collected in EDTA (1 mg of EDTA per 1 ml blood) or heparin and submitted on wet ice, or refrigerated but not frozen. (For virus isolation.)
   b. Serum from onset of disease and at death or recovery. (For serology.)
2. Tissues (for virus isolation, refrigerated but not frozen.)
   a. Spleen.
   b. Lymph nodes.
   c. Adrenal.
   d. Thyroid.
3. Tissues. (For histopathology, 1/4" slices fixed in 10 volumes of 10% neutral buffered formalin.)
   a. Kidney.
   b. Spleen.
   c. Liver.
   d. Adrenal.
   e. Lymph nodes.

RINDERPEST (NOTE: SEE APPENDIX 2)

1. Blood.
   a. Whole blood, heparinized, frozen. (For virus isolation.)
   b. Serum, 20 ml, frozen from acutely ill and convalescent animals. (For serology.)
   c. Blood smears; 6 slides air dried and fixed in absolute alcohol. (For differential diagnosis.)
2. Tissues. Collected aseptically, frozen. (For virus isolation.)
   a. Spleen.
   b. Lymph nodes (mesenteric, draining affected areas).
3. Tissues, not more than ¼" thick, fixed in 10 tissue volumes of 10% neutral buffered formalin. (For histopathology.)
   a. Mouth and tongue lesions.
   b. Spleen.
   c. Liver.
   d. Lung.
   e. Kidney.
   f. Lymph nodes (draining affected areas).
   g. Sections from intestines.
4. Fecal sample. (For differential diagnosis.)
5. Formulas. See appendix 1.
6. History to be submitted with specimens. See appendices 3 and 5.

*Some laboratory personnel recommend cutting tissues 3 mm thick and fixing in 50X.

SWINE VESICULAR DISEASE: See FMD.

VESICULAR EXANTHEMA OF SWINE: See FMD.

VESICULAR STOMATITIS: See FMD.

SHEEP POX

1. Blood.
   a. Whole blood taken during febrile period and submitted frozen.
   b. Serum from the acute and convalescent stages.
2. Lymph nodes and lesions.
   a. Submit frozen.
   b. Submit in buffered glycerin.
   c. Submit small sections frozen on metal foil for FA.

APPENDIX 1

1. OPG or OCG Oxalate Phenol Glycerine Solution.

   Potassium oxalate..... 5 gm

   Carbolic Acid (Phenol)..... 5 ml

   H_2O.....500 ml

   Glycerine.....500 ml

2. Glycerol (Glycerin) Buffer, pH 7.2 (Phosphate Buffered Glycerin).

   a. Monobasic Sodium Phosphate (NH_2PO_4,$2H_2O$)

      31.2 g per L, distilled H_2O

   b. Dibasic Sodium Phosphate (Na_2HPO_4,7 H_2O)

      53.65 g per liter of distilled H_2O

   Add 28 ml solution A = 72 ml solution B to an equal volume of glycerol.

3. Ten Percent Neutral Buffered Formalin.

   Monobasic Sodium Phosphate ($NaH_2PO_4 \cdot 2H_2O$)..... 4.0 gm

   Dibasic Sodium Phosphate (Anhydrous Na_2HPO_4)..... 6.5 gm

   Distilled H_2O ..... 900 ml

   Formalin (40%)..... 100 ml

   Use a volume of above at least 10 times the volume of tissue. (For a few tissues, some personnel recommend 50X.)

4. Disodium Ethylenediamine Tetraacetate (EDTA). Use 0.5 to 1 mg per 5 ml of blood as an anticoagulant.

5. Heparin. Use 0.1 to 0.2 mg per ml of blood as an anticoagulant.

6. Sodium Citrate Anticoagulant.

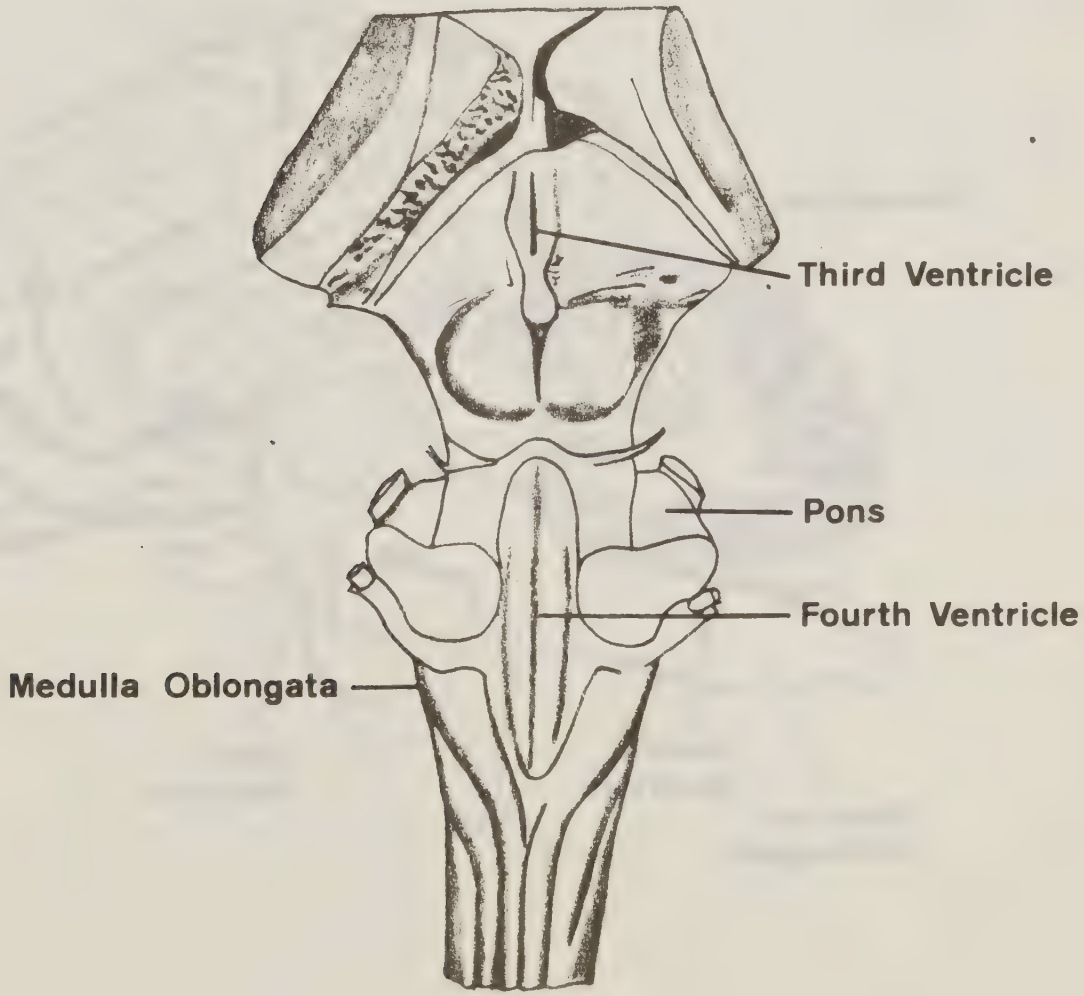
   Sodium citrate..... 10.0 gm

   H_2O (dist.)..... 100.0 ml

   Sterilize by autoclaving: 1 ml per 10 ml blood.

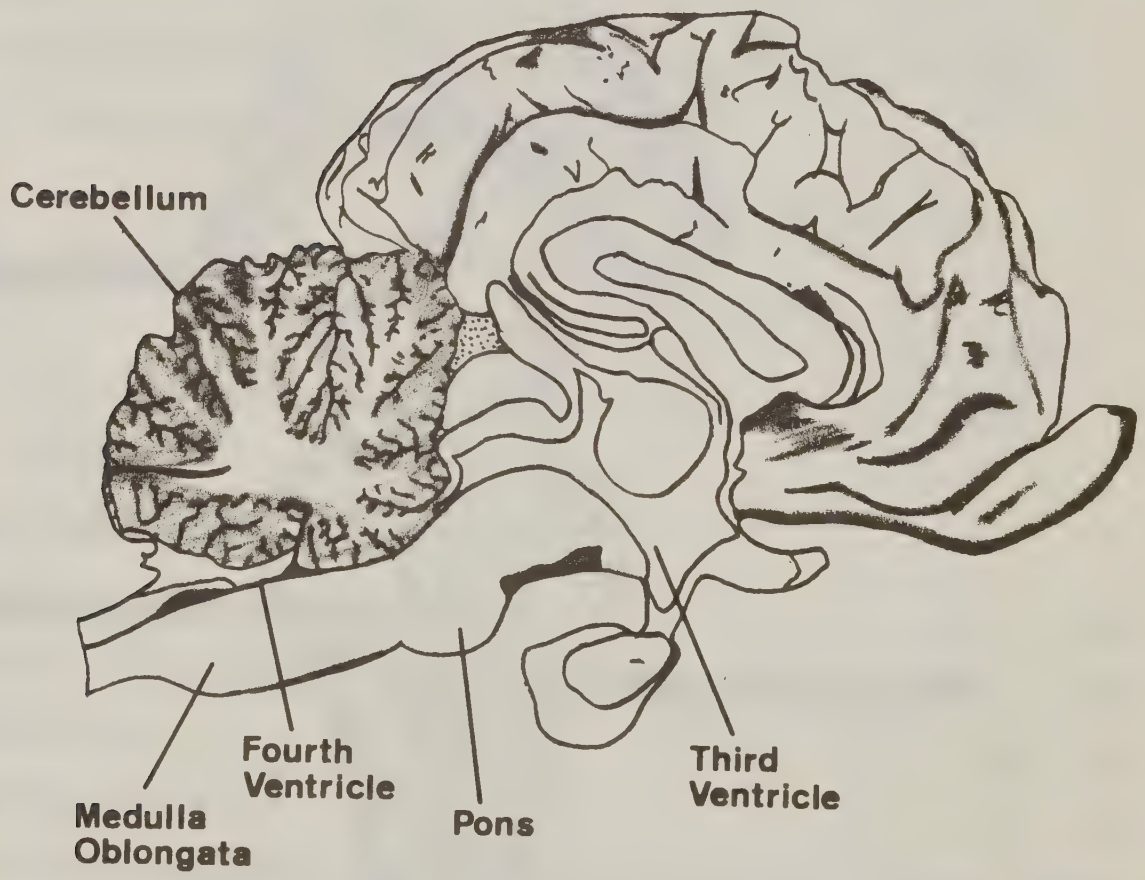
Appendix 1a

DISSECTION BRAIN STEM AND BASAL GANGLIA OF HORSE BRAIN (DIAGRAMATIC)



Appendix 1b

SAGITAL SECTION OF OX BRAIN (DIAGRAMATIC)



APPENDIX 2

EXCERPTS FROM "EMERGENCY ANIMAL DISEASE ERADICATION GUIDE" (The"Red Book") DEALING WITH LABORATORY TESTS FOR CONFIRMATION OF AN EMERGENCY ANIMAL DISEASE

PART B-DIAGNOSIS OF A SUSPECTED EMERGENCY ANIMAL DISEASE

1. Laboratory Tests for Confirmation of an Emergency Animal Disease

Laboratory tests for emergency animal diseases will be conducted at the Plum Island Animal Disease Center.

2. Notification

Any and all suspicions of the existence of an emergency animal disease should be reported to the appropriate ANH Assistant Director, or a member of the Emergency Animal Diseases Staff. The Director or designated representative of the ANH Division will consult with the Director or designated representative of the Veterinary Sciences Research Division (VSR) to determine whether diagnostic specimens should be sent to the Plum Island Animal Disease Center (PIADC) for diagnosis. The Director of ANH will advise the diagnostician whether to proceed with the collection and submitting of specimens to PIADC for diagnostic purposes. If submissions are to be made to PIADC, the Director or designated representative of VSR will immediately notify the PIADC authorities and the Director or designated representative of ANH will arrange for a courier to take the diagnostic specimens to PIADC.

In no instance should diagnostic specimens be submitted to the Division or any other place until requested by the ANH Director or his authorized representative (ANH Division Memorandum 587.1 dated April 15, 1966). The Director of ANH should be notified of (1) the airline, (2) the flight number, and (3) the expected arrival time and name of the courier.

3. Submission of Diagnostic Specimens to the Laboratory

The packaged specimens must be in the custody of a courier at all times. The selection of the courier and the mode of travel will be determined by the Director of ANH. The courier will travel by the fastest means to one of the transportation terminals in New York City, where he will be met by a representative of PIADC. Other methods of transportation of specimens from the terminal to PIADC may be used by mutual agreement between appropriate representatives of the two Divisions. If the PIADC officials wish to have the courier remain while the diagnosis is being made so that he can give them additional information as needed, ANH will make such agreements.

4. Progress of Investigation

The Director of ANH will keep the Director of VSR advised on the current status of field developments. At the time the specimen are delivered, the courier will provide PIADC with a written report, as complete as may be available, of the history and current status of the investigation.

The Director of VSR will keep the Director of ANH advised of the results of the tests in progress at PIADC.

5. Address of Plum Island Animal Disease Center

Plum Island Animal Disease Center
Post Office Box 848
Greenport, L.I., New York 11944
Telephone: A.C. 516-323-2500

6. Addresses of ANH and VSR Personnel

Dr. R.S. Sharman, Director
Animal Health Division
Agricultural Research Service
U.S. Department of Agriculture
Hyattsville, Maryland 20782
Office Telephone: A.C. 202 DU 8-8612
Home Telephone: A.C. 301 262-1844

Dr. R.E. Omohundro, Assistant Director
Animal Health Division
Agricultural Research Service
U.S. Department of Agriculture
Hyattsville, Maryland 20782
Office Telephone: A.C. 202 DU 8-8612
Home Telephone: A.C. 703 KE 6-8154

Dr. J.J. Callis, Director
Plum Island Animal Disease Center
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Greenport, L.I., N.Y. 11944
Office Telephone: A.C. 516 323-2500
Home Telephone: A.C. 516 765-2259

Dr. J.H. Graves, Assistant Director
Plum Island Animal Disease Center
Post Office Box 848
Greenport, L.I., New York 11944
Office Telephone: A.C. 516 323-2500
Home Telephone: A.C. 516 765-2986

Dr. A.H. Dardiri, Leader, Diagnostic Investigations
Plum Island Animal Disease Center
Post Office Box 848
Greenport, L.I., New York 11944
Office Telephone: A.C. 516 323-2500
Home Telephone: A.C. 516 765-2763

7. Specific Tissues or Specimens Required for Laboratory Diagnosis of Vesicular Diseases (FMD, VE, and VS)

When a vesicular condition is suspected, specimens for laboratory confirmation should include a minimum of 10cc. of serum, 5 grams of vesicular lesion tissue preserved in phosphate buffered glycerin, a probang specimen of 10cc. oesophageal-pharyngeal (O-P) fluid in 10cc. of phosphate buffered glycerin and 10cc. of whole blood. If vesicular fluid is available it should be collected separately, frozen and submitted with other specimens. All available tissue should be submitted if 5 grams of tissue cannot be harvested. Probang (O-P) fluid should be submitted in all cases, even though accompanied by voluminous vesicle harvest from the same animal.

a. Blood or sera for serology.-A blood sample should be taken from individually identified infected animals during the initial investigation. A second set of serum samples should be taken 2 to 3 weeks later from the same animals. If at the time of the initial investigation it is determined that 2 to 3 weeks have already elapsed since any animal was in the acute stage the second set of serum will not be necessary.

Serologic tests can be made on the serum. Two 10 cc. samples of blood from each animal to be tested should be collected aseptically. Samples should be taken from all, or a representative number, of those outwardly affected as well as from those that are not affected. Aseptically separate the clot from the serum and discord clot. Seal the serum container securely to prevent leakage. Refrigerate or freeze the serum until time to ship, and ship frozen with dry ice. Tubes to be frozen should not be filled more than halfway and should be frozen in a slanted position. Obtain permission from the Director of ANH, appropiate Assistant Director, or member

(continued on the following page)

of the Emergency Animal Diseases Staff before shipping. The diagnostician will be responsible for proper disposition of any unused blood, serum or specimen material.

b. Blood for virus isolation—Blood may be used for virus isolation in vesicular disease diagnosis, although the virus concentration is usually much higher in the epithelium that covers the vesicle. It has been found that FMD virus may be isolated from blood collected during the acute stages and especially the febrile stages (104°-106° F.) of the disease. Whole blood should be collected, frozen, and submitted to PIADL.

c. Tissue for virus isolation.—Select animals showing temperature elevations. Identify each animal from which material or serum is collected.

Remove vesicular covering from unruptured or freshly ruptured vesicles on the muzzle, tongue, buccal mucous membrane, coronary band, interdigital space, or teats. When lesions are covered with mud or debris, clean with clear water before harvesting specimens. Check the pH of the water with litmus paper before you use it. If the water in the area is highly alkaline or acid, do not use it since it could inactivate the virus.

It is essential to provide tissue to the laboratory in cases suspected of being vesicular. When vesicular material from unruptured or freshly ruptured vesicles is not available, every effort should be made to harvest tissue from the periphery as well as the crater of the lesion. Use small scissors or scalpel to trim material from the periphery or, if necessary, scrape the area, or several lesion areas, to obtain sufficient material for a diagnostic test. If necessary, consideration should be given to buying the animal so that sufficient material may be obtained. In such cases it may be necessary to sacrifice the animal and excise the entire lesion. Include apparently normal tissue adjacent to the lesion. Lymph nodes may also be used for virus isolation. Large nodes should be trimmed to fit in the vial.

Place material in a vial of 50 percent phosphate buffered glycerin. There should be two parts of this solution for each part of vesicular material. Freeze or refrigerate sample until time to ship, and ship frozen.

Do not fill vials more than half full. The diagnostician should keep some material (if sufficient material is available) buffered and frozen, or refrigerated for reference if needed. The diagnostician will be responsible for proper disposition of any unused specimen material.

Seal vials with moisture repelling tape or sealing wax to prevent leakage and prevent CO_2 from the dry ice gaining entry into the vial. CO_2 will change the pH of the liquid in the vial. CO_2 and H_2O will yield carbonic acid, which can inactivate the virus in the sample and render it useless.

Label vials properly. Identify source if the material is collected from more than one animal. Include Owner's name and address, species, date of harvest, and type of material collected.

Individually wrap each vial in sufficient absorbent cotton to assure absorption of all fluids in event of breakage.

- Place vial or vials in double mailing tubes.
- Include a copy of report in the mailing tube.
- Dip both the inside mailing carton and the outside mailing carton in 2 percent lye solution.

During all stages of packing, the material should be kept refrigerated.

Place mailing tube in shipping container, packed with dry ice. The material will be transported in accordance with instructions from the Director of ANH. If transported by air, the name of the airline, the flight number, and the flight schedule shall be furnished to the Director of ANH.

d. Tissues for histopathology.— None are necessary.

e. Procedures for collecting probang specimens from bovines and from small ruminants. A probang is defined as a flexible rod with a ball, tuft, or sponge at the end, used in diseases of the oesophagus or larynx. The instrument may be used for applying medication to these areas. The probang for collecting oesophageal-pharyngeal (O-P) fluid has been modified with a cup attached to the end. The cup is inserted into the oesophageal-pharyngeal region to collect saliva and tissue which may contain foot-and- mouth disease (FMD) virus. The virus of FMD can become established in the surface epithelium of the pharyngeal region and multiply, creating a carrier status. This can occur in vaccinated animals exposed to virus, animals recovered from infection, and nonimmune animals exposed to small quantities of virus. Carrier animals may or may not be serologically positive.

(1) The following techinque should be used to collect probang specimens from bovines:

(a) The animal should be well restrained, preferably in a chute. Nose tongs may or may not be used, depending upon the docility of the animal.

(b) The head of the animal should be held in a straightforward position and slightly elevated. With docile animals, an assistant may stand to the animal's right side, restrain the animal, and elevate the head with his left arm.

(c) The operator should face the animal, standing slightly to the left side of the animal's head. (Left-handed operators may prefer to stand on the right.)

(d) The probang cup should be inserted into the left side of the animal's mouth and pushed over the dorsal prominence (hump) of the tongue; with gentle pressure, the animal will swallow the cup. The cup should be allowed to pass into the oesopgagus, and by moving it back and forth it can be seen or palpated along the side of the neck, assuring that the cup is in the oesophagus and not in the trachea.

(e) The cup should be moved back and forth in the oesophageal-pharyngeal region with some vigor five or six times.

(f) The cup may be withdrawn with a firm, steady pressure, resulting in little or no injury to the animal. As the cup is withdrawn, care should be taken to keep the cup in an upright position to prevent spillage.

(g) Similar to people, many animals have a dryness of their month and throat when excited. This will influence the amount of material collected with each passage of the probang, which under conditions where the cattle are not greatly excited may average about 3 cc. Experience has shown that rinsing the mouth with water or drenching with a bottle of water prior to and between probang passages does not reduce the chances for isolation of virus from the samples. Saliva *per se* is not what is wanted in the specimen, since it usually contains very little virus. The desquamated epithelial cells and mucous of the pharyngeal and upper oesophageal regions is the material most likely to contain virus. This material is loosened and collected in the cup by vigorous movement of the probang. Saliva and residual water from the mouth mainly serve as the suspending vehicle for the cells and mucous and make it easier to pour the samples out of the cup. The probang should not be used in such a way that small hemorrhages are produced, since blood in the specimen is undesirable. If the animal regurgitates or if excessive food material is contained in a specimen, the specimen should be discarded, the animal's mouth flushed with water, and additional collections made. Usually, the minimum collection of 10 cc or more of O-P fluid can be obtained by three passages of the probang. It is generally not desirable to exceed three passages of the probang because the irritation may result in bloody specimens.

(h) a) After each passage of the probang, the specimen in the cup should be poured into the specimen container until 10 cc or more of specimen has been obtained. It is important that immediately after obtaining the desired amount of specimen an equal volume of the phosphate buffered glycerin should be added to the specimen and the contents of the specimen container then should be vigorously shaken for about a minute. The buffering action of the phosphate buffered glycerin will neutralize the saliva and mouth rinse water in the specimen. The indicator dye in the phosphate buffered glycerin solution gives a purple color when alkaline, red near neutral and orange to yellow when acid. A shaken specimen with a red to slightly purple color is desirable. If when shaken, specimen have a yellow color it may indicate that the water used is too acid. Then further collections should be done utilizing another source water. Likewise, if the water is too alkaline another source should be used.

b) If the specimen in the probang cup is so thick and tenacious that it will not pour out, a measured amount of phosphate buffered glycerin may be placed in the cup and stirred with a clean large gauge needle until the specimen can be poured into the specimen container. The amount of phosphate buffered glycerin added should not exceed the ratio of approximately equal parts of specimen and solution. Specimens of this kind indicate that the animal's mouth and throat are too dry. The animal's mouth should be rinsed or a drench should be used before the next probang passage is made.

c) A special probang is being developed for calves. It is similar in design to the regular probang, except that the cup is smaller in diameter. Do not attempt to take probang samples from young calves with the regular-size cattle probang because it is too large. Use the probang designed for small ruminants on calves until the calf probang is available.

(i) Freezing may be readily accomplished by using a mixture of dry ice and alcohol. Chunks of dry ice, approximately 2 or 3 inches square, should be placed in a pan, approximately 12 inches square by 4 inches deep. Ninety-five percent ethyl alcohol should be poured into the pan to a depth of 2 to 3 inches. Considerable bubbling and sputtering will occur when the alcohol and dry ice are mixed. Rubbing alcohol may be used, but the water content will cause a slush to form, which is not as good as 95 percent ethyl alcohol for freezing the material.

(j) The specimen container may then be partially immersed in the alcohol and gently rotated. The material will freeze quickly. After the specimen is frozen, each container should be checked for cracks and placed in a double mailing tube with sufficient cotton to prevent breakage in transit. The entire unit should then be placed in a styrofoam shipping container with dry ice.

The probang for small ruminants differs from the bovine probang in size and shape. This probang has two small cups with the concave surfaces facing each other. Careful examination will reveal that the diameter of the top cup is slightly larger than the bottom cup. This facilitates collection of O-P tissue and fluids on the downward stroke of the probang. The smooth convex surface of the top cup prevents the instrument from cutting or damaging the tissues.

(2) The following technique should be used to collect probang specimens from small ruminants:

(a) The animal must be well restrained. An assistant may straddle the animal's neck and grasp one jaw in each hand forcing the mouth open.

(b) The operator should face the animal and insert the probang directly down the center of the tongue to the dorsal prominence (hump). With gentle pressure against the dorsal prominence, the animal will swallow the probang.

(c) The probang should be moved back and forth in the oesophageal-pharyngeal region with vigor five or six times. Tests in sheep have shown that the highest concentration of the virus is in the tonsillar region.

(d) The probang may be withdrawn with a firm steady pressure. If difficulty is encountered in removing the probang, pull gently until the animal stops swallowing and releases the cup.

(e) The amount of material collected from small ruminants will be much less than O-P fluids collected from bovines (0.2 ml. per passage). The probang should be passed at least 3 or 4 times and the material collected placed in 5.0 ml. of phosphate buffered glycerin.

(f) Each collection should be immediately transferred to the specimen container. This may be accomplished in either of two ways. Place the cup in the specimen container of phosphate buffered glycerin and agitate, or use a syringe to flush the material from the probang cup into the container.

(g) The specimen should be sealed, frozen, and packed for shipment as described for the bovine.

(h) The probang may be disinfected between animals with 2 percent acetic acid, or vinegar, or 2 percent sodium hydroxide. Special precaution must be exercised to assure that the probang is thoroughly rinsed with clean water before reuse. A small amount of disinfectant may alter the pH and destroy any virus present.

f. Probang procedures are not necessary in swine at this time. The carrier state of FMD has not yet been demonstrated in swine; however, research is continuing.

8. Specific Tissues or Specimens Required for Laboratory Diagnosis of Rinderpest

Virus is present in all fluids and tissues during illness. Virus can be obtained at highest titer from splenic tissue on the second morning of temperature over 104° F. A titer of 10^4 to 10^5 may be obtained at that time.

a. Blood or serum for serology. Blood serum obtained during the first 6 weeks of convalescence will contain complement-fixing antibodies. If possible, paired sera should be obtained from each animal, that is, one serum early in the acute phase and another 2 weeks later during convalescence. For diagnosis by complement-fixation during the early acute phase, it is necessary to use tissue, preferably lymph nodes, for the preparation of antigen against known positive rinderpest antiserum.

Serum obtained during convalescence or later will contain a neutralizing antibody of high titer. A generous quantity (20 ml.) of such serum should be obtained, frozen, and held for eventual use in serum-virus neutralization tests.

b. Blood for virus isolation.—The virus titer of blood is highly variable; therefore, blood is not recommended for virus isolation.

c. Tissues for virus isolation.—Spleen and mesenteric lymph nodes collected at the height of fever are the tissues of choice for virus isolation from rinderpest infected cattle. The tissues should be collected aseptically, placed in a sealed container, and frozen for shipment. Special precautions should be taken to prevent the container from leaking or CO_2 from the dry ice entering the container.

d. Tissue for histopathology.—Tissue for histopathology should include various areas of the mouth and tongue that present lesions, each part of the digestive tract, each of the parenchymatous organs, and representative lymph nodes. Tissue should be obtained early in the course of the disease at a time when the early lesions are grossly evident. Tissue should not be more than one-fourth of an inch in thickness and should include adjacent normal tissue as well as the lesions. Tissues should be fixed in 10 times their volume of neutral 10 percent formalin.

9. Specific Tissues or Specimens Required for Laboratory Diagnosis of Teschen Disease

a. Blood for serology.—The virus neutralization test conducted in primary pig kidney cell culture is used for the detection of Teschen Disease virus.

Blood samples should be collected from diseased animals upon initial investigation. A second set of samples should be taken 2 or 3 weeks later from the same animals. Aseptically collect the blood, separate the serum, seal the vial, and refrigerate or freeze it until time to ship.

b. Blood for virus isolation.—Blood is unsatisfactory as a source of virus because the period of viremia is short, transient, and variable.

c. Tissues for virus isolation.—The virus tends to localize in the brain and spinal cord; consequently, it is well to take portions of the midbrain, cerebellum and medulla, and portions of spinal cord from cervical through lumbar regions for virus isolation attempts. These tissues should be pooled and frozen until they can be prepared for passage in cell culture and susceptible baby pigs. One procedure for preparing brain specimens is to incise the midbrain, cerebellum, and medulla through midline, and freeze half for virus isolation and place half in formalin for histopathological examination.

d. Tissues for histopathology.—Microscopic lesions observed in the midbrain, cerebellum, medulla, and spinal cord are of significance in the diagnosis of Teschen disease; consequently, it is important to obtain these tissues for histopathology. Tissues should be obtained while the temperature is high, during the first 2 or 3 days of paralysis; by that time the lesions should be well developed and easier to contrast with those found in the early stages of hog cholera. The most differentially specific lesions are found in the cerebellum and spinal cord. Portions of the brain and spinal cord selected for histopathological examination should be fixed while intact. To rule out other diseases, take tissues from all of the body organs, including several visceral and peripheral lymph nodes, for histologic study. Tissues such as kidney, bladder, epiglottis, and spleen, which normaly present lesions in animals affected by hog cholera, should be examined with care and any suspicious lesion included in the tissue sent in for histopathologic perusal.

Tissue for histopathological studies, other than brain and spinal cord, should not be more than 1/4 inch thick and should be immediately preserved in 10 times their volume of neutral 10 percent formalin.

10. Specific Tissues or Specimens Required for Laboratory Diagnosis of African Swine Fever

a. Blood or serum for serology.—If any of the animals suspected of being infected with African swine fever survive, a generous quantity (50 cc. minimum) of the serum should be collected and frozen.

b. Blood for virus isolation.—Blood taken from the animal any time after the temperature is well established should contain large amounts of virus. The ideal time to collect is at the peak of temperature. Such blood specimens should be frozen.

c. Tissues for virus isolation.—Generous portions of spleen, lymph nodes, and kidney should be harvested and frozen for submission to the laboratory. The spleen taken a day or two after the temperature peak has been reached will contain large quantities of virus. After the disease is well established, the virus will be present in all tissues and fluids until the death of the animal. Those few chronic cases that persist for some weeks or months may continue to carry the virus in blood.

d. Tissues for histopathology.—In a typical case, the animal will die after 7 or 8 days of above normal temperatures. Tissues should be taken when lesions are well developed during the last 24 to 36 hours before the expected time of death of the animal or just at the time of death. Samples of spleen, tonsil, liver, adrenal, and kidney should be collected. It is important to obtain the brain and several visceral and body lymph nodes. Tissues should be taken and prepared in even slices one-fourth of an inch thick and should include the lesion

and adjacent normal tissue. The brain should be fixed whole. All tissues should be fixed in 10 times their volume of neutral 10 percent formalin. The amount of formalin may be reduced after fixation before shipment; if so, fresh formalin should be used for shipping the specimens.

11. Specific Tissues or Specimens Required for Laboratory Diagnosis of Rift Valley Fever

a. Blood or serum for serology.—Serum should be obtained from some normal animals and from animals in the earliest stage of illness, for comparison with serum from convalescent animals in the same herd.

b. Blood for virus isolation.—Blood will contain virus during the acute phase of illness. Such blood should be preserved by freezing.

c. Tissues for virus isolation.—Virus is present in all tissue during the acute phase. Liver or spleen should be collected and frozen.

d. Tissue for histopathology.—Tissue for histopathology should include tissue from each of the parenchymatous organs. Care should be taken in obtaining tissue from the liver to include both normal and grossly involved areas. Fix all tissues in 10 times their volume of neutral 10 percent formalin solution.

12. Specific Tissues or Specimens Required for Laboratory Diagnosis of Bovine Pleuropneumonia

a. Blood or serum for serology.—Serological diagnosis by complement-fixation (CF) or agglutination tests is especially helpful in mild or chronic cases and will detect late acute or chronic phase cases. When possible, serum for tests should include paired samples from the same animal; i.e., the first sample collected as early as possible after onset of the disease and the second sample 2 or 3 weeks later. Herd samples should include sera from some normal, acute, chronic, and convalescent cases. All should be labeled accordingly and when possible bear reference to clinical symptoms or necropsy lesions.

Serum should be aseptically separated from the clot in field laboratories, and frozen for submission to the laboratory.

b. Blood for isolation of causative agent.—The causative agent may be recovered from blood or serum until the lung lesions encapsulate. Take 20 cc. of blood and ship refrigerated. DO NOT FREEZE.

c. Tissues for isolation of causative agent.—Aseptically aspirate 10 cc. (or more if available) pleural fluid and lung exudate from an acute pneumonic area. Aseptically cut two or three blocks of tissue, approximately one-inch square, from the active edge of acute lesions. Ship refrigerated. DO NOT FREEZE.

d. Tissues for histopathology.—Take 1/4 inch-thick slices from the lungs. Include in the slices normal tissue adjacent to the lesions. When no gross lesions are evident, take representative 1/4 inch-thick slices from different sections of the lung. If there is a possibility of Malignant Catarrhal Fever, include the intact eye, conjunctiva, and portions of the brain, including the floor of the fourth ventricle. Fix all tissues in 10 times their volume of neutral 10 percent formalin solution.

13. Specific Tissues or Specimens Required for Laboratory Diagnosis of African Horsesickness

a. Blood or serum for serology.—Collect blood aseptically from recovered or immunized horses to test for the presence of complement-fixing or neutralizing antibodies. Remove the clot and preserve the serum by freezing.

b. Blood for virus isolation.—Blood collected at the height of fever is preferred for virus isolation. It should be collected in equal volumes of O.P.G. (an anticoagulant preservative)[1] and refrigerated. (use of dry ice is not recommended.)

c. Tissue for virus isolation.-Spleen should be collected and kept frozen until arrival at the laboratory. Any other tissues that appear abnormal should be frozen and included.

14. Specific Tissues or Specimens for Laboratory Diagnosis of Other Emergency Diseases

When an emergency disease other than those listed in this section is suspected, the diagnostician should contact the ANH Director, appropriate Assistant Director, or member of the Emergency Animal Diseases Staff regarding collection and shipment of specimens.

15. Diagnosis of Animal Inoculation

If it is necessary to make animal inoculations for a differential diagnosis, such inoculations should be made on the premises where the disease is found. When the test is being conducted in an area where one of the diseases being differentiated may be endemic, caution should be taken in selecting diagnostic animals to guard against the use of possibly exposed and therefore immune animals. The diagnostic animals should be brought from an area that is a considerable distance (100 miles or more depending on history of disease conditions at source) from that where the case being investigated exists. The animsls for the test must include a minimum of two each of the species susceptible to the diseases being differentiated. Any deviation in the number and species of animals used must have the approval of the ANH Division Director's office.

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[1] O.P.G. Solution:

Phenol	5 g.
Potassium oxalate	5 g.
Glycerin	500 ml.
Distilled water	500 ml.

16. Policy for Procurement and Disposal of Diagnostic Animals

In some cases, it may be desirable to arrange for obtaining diagnostic animals before the special diagnostician arrives at the scene of investigation. Preferably, the State officials will arrange for the procurement of diagnostic animals, since in many instances, State officials can handle matters of this kind more informally than the Federal Government. If it is not possible for the State to procure the diagnostic animals, the Federal Government can arrange for their purchase.

Purchase of diagnostic animals will be governed by Administrative Memorandum No. 210.4, dated October 2, 1963. Purchase of the animals can be made on Form SF-44. If the seller demands cash for the animals, a signed receipt should be secured, and claim should be made for reimbursement on the employee's travel voucher in accordance with the Standardized Government Travel Regulations.

Delegation of sales authority for disposal of noninfected diagnostic animals should be obtained from the appropriate servicing Administrative Office in accordance with the provisions of Paragraph III of Administrative Memorandum 220.3.

Part H—Disposal

1. Disposal by Burial

Burying is the preferred method of disposal and should be used whenever practical. Digging the disposal trench should begin as soon as possible after confirmation of the diagnosis. The site should be on the infected premises or as close as topography permits. When selecting a burial site, consider underground cables, water or gas lines, septic tanks, water wells, etc. If possible, choose an area away from public view.

a. Contracts for disposal.—Contracts for the use of heavy equipment should include dimensions of the trench, cubic yards of material to be moved, price per yard, charges for blasting or other special techniques, and provision for trench filling. Administrative procedures for contracting services are contained in Section II—Administrative Procedures.

b. Trench dimensions.—A burial trench should be at least 7 feet wide and 9 feet deep. At this depth, 14 square feet of floor space is required for each bovine carcass (5 mature hogs or sheep equal one bovine carcass). It may be desirable to dig deeper (12 to 20 feet) and wider trenches or pits, depending on soil conditions and types of digging equipment available. For every additional 3 feet in depth, the number of animals per 14 square feet of floor space can be doubled. Refer to Section III, Part G. paragraph 3, Methods of Depopulation, for additional information.

c. Disposal of feed, milk, manure, and miscellaneous items.—Contaminated feed, milk, manure, and other items should be placed in the trench with the carcasses and covered with at least 6 feet of soil. The trench site should be mounded over and the area neatly graded. Do not pack the trench. Decomposition and gas formation will crack a tightly packed trench causing it to bubble and leak fluids. Refer to Section III, Part I, paragraph 3,h, for instruction on disinfection of milk.

2. Disposal by Burning

Burning carcasses is difficult and expensive in terms of labor and material. Burning should be used for disposal only when burial is not feasible because of conditions such as high water table or excessive rock, or for public health reasons such as being too close to municipal water supplies.

A holding pen for confining animals prior to euthanasia should be available near the burn site. In some instances, farmyards and existing holding pens may be adapted for this purpose. In other cases, new pens may have to be constructed.

a. Selection of burn site.—The burn site should be selected with care and should be readily accessible to heavy vehicles hauling materials. A flat area away from public view is desirable. Keep the fire well away from buildings and hay, straw, or feed stacks. Don't build the fire near overhead electric or telephone cables. Avoid building the fire over shallow underground water pipes or gas mains. The prevailing wind direction should be considered to prevent unnecessary quantities of smoke and objectionable odors from blowing down on farm buildings or across public roads. The fire will burn better if constructed at a right angle to the prevailing wind.

b. Burning procedures.—A burning operation consists of elevating the carcasses on a platform constructed of incendiary materials (oil, wood, coal, straw, old tires, etc.). It will often be difficult to obtain sufficient quantities of suitable incendiary materials. The individual in charge of building the fire must use ingenuity in acquiring materials and putting them to optimum use. Until carcasses are destroyed, the fire should be guarded to avoid dissemination of infected material by predatory animals or birds. The fire will have to be tended and rearranged periodically as it progresses. A small bulldozer or a tractor with scoop is useful for this purpose. The straw bale platform method of carcass disposal by burning is described below.

Fuel Requirements

- Straw or hay: Allow 3 bales per cattle carcass. Contaminated straw or hay can be used in fire preparation. Additional quantities can be purchased from the farmer or local suppliers.
- Heavy timber: Allow 3 pieces (approximately 8 feet long by 1 square foot in cross section) per cattle carcass. Railroad ties or bridge timbers make ideal material. If smaller dimension materials such as fence posts or cord wood are used, proportionately more pieces will be needed.
- Old tires: Allow 4 or 5 tires per cattle carcass.
- Kindling wood: Allow 50 pounds per cattle carcass. This material may be obtained from wrecking companies, farm wood piles, saw mill slab piles, etc.
- Coal: This should be of good quality and in large lumps (6 inches to 8 inches diameter preferable; avoid fine coal). Allow 500 pounds of coal per adult cattle carcass. Proportionately less is required for young stock.

  When goats, sheep, or swine are burned with cattle, they may be placed on top of the cattle carcasses at the rate of two animals for each cattle carcass without additional fuel. Over this rate, or when goats, sheep, or swine are burned alone, allow 100 pounds of coal per animal.
- Liquid fuel: Waste oil, furnace oil, or diesel fuel should be obtained in sufficient quantity to thoroughly soak the other materials before the fire is lighted. A minimum of 1 gallon per cattle carcass is required. A reserve supply of fuel oil should be held in case difficulty in burning is encountered. Caution, do not use gasoline.
- Estimation of animals:

  1 adult cow or bull = 1 C
  5 adult swine = 1 C
  5 adult sheep = 1 C

  Reduce all animals to number of C's.
- Length of fire: One yard per C (2 pigs, 2 goats, or 2 sheep can be layered on top of each C).
- Amount of material per C:

Straw	= 3 bales per C
Heavy timbers (8 ft. long x 1 ft. sq.)	= 3 per C
Kindling wood	= 50 lb. per C
Tires	= 4 per C
Coal	= 500 lb. per C
Fuel Oil	= 1 gal. per C

Example:

500 cattle	= 500 C
1,000 swine	= 200 C
700 sheep	= 140 C
Total	840 C

Reduce 840 C by 200 C since 2 swine or 2 sheep carcasses may be added for each cattle carcass without additional fuel. 840 C – 200 C = 640 C.

Stake out fire line 640 yards long. Line can be divided into 2 or 3 separate lines.

Straw–3 bales per C	= 1,920 bales
Heavy timber–3 per C (increase if small timbers are used)	= 1,920 timbers
Kindling wood–50 lb. per C	= 16 tons
Tires–4 per C	= 2,560 tires
Coal–500 lb. per C	= 160 tons
Liquid fuel–1 gal. per C	= 640 gallons

Fire Preparation

(See fig. 3, "Disposal of Carcasses by Burning")

DISPOSAL OF CARCASSES BY BURNING

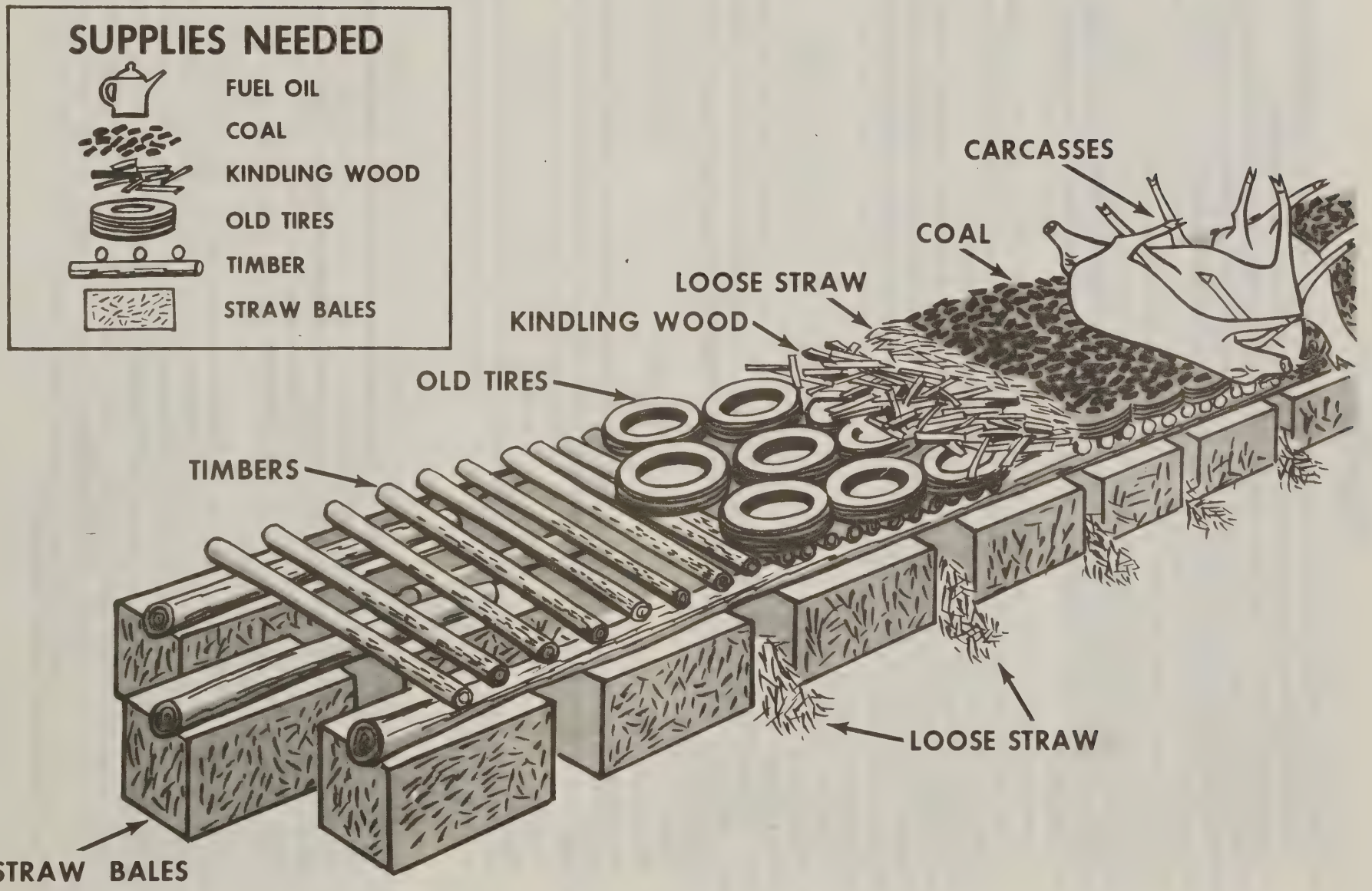

Figure 3

- Select a site and stake out the area of the firebed, allowing 3 feet of length for each adult cattle carcass.
- Lay three rows of straw or hay bales lengthwise along the line of the firebed.
- Allowing 3 feet run per adult bovine carcass, lay the rows approximately 12 inches apart with 12 inches between each bale in a row.
- Push loose straw into the space between the bales.
- Place the large timbers lengthwise on top of each row of straw.
- Distribute the remaining large and medium-sized timbers across the firebed with 6 to 12 inches of space between timbers.
- Next, place the old tires and small kindling wood on the firebed.
- Spread loose straw over the wood and tires.
- Spread the coal evenly (at the rate of 500 lb. per yard) over the wood and tires to make a level bed. A front-end loader is essential for spreading the coal.
- Place carcasses on the firebed. Position them on their backs with feet in the air alternately, head to tail. This can best be done with mechanical lifting equipment (front-end loaders, draglines, trenchers) and chains.
- Place loose straw over the carcasses and stuff into all the spaces between carcasses. Pour or spray liquid fuel (Caution: do not use gasoline) over the pyre with buckets or sprinkling cans. If a pump is available, spray the fuel on. Start the fire along the entire length of the pyre. A torch that will burn for several minutes is recommended for starting the fire.

  If weather conditions are favorable with a good steady wind, the bulk of the carcasses should be burned within 48 hours. It will be necessary to tend the fire, stirring it occasionally, and replacing carcass pieces that drop off. Additional fuel may need to be added. When all the carcasses have been completely burned and the fire has died out, the ashes should be buried and the area cleaned up, graded or plowed, and prepared for seeding.

3. Disposal by Selling for Slaughter

In some disease conditions, salvage by slaughter, under supervision, may be permitted. Special attention must be given to methods and equipment used for transporting such animals to slaughter. Salvage procedures should be developed by the inspection and diagnosis section of the State EADEO.

4. Other Selected Disposal Methods

Upon recommendation of the ANH Veterinarian in Charge and the State Livestock Sanitary Official, the ANH Division Director may provide for other disposal methods, such as rendering, in extraordinary circumstances. (Reference: Title 9, CFR, Part 53.4.)

Part M—Procedures on an Infected Premises

1. Guideline for Diagnostician Assigned To Investigate Reported Suspicious Cases

a. When a suspected case of foot-and-mouth disease is reported to the EADEO field unit office, the owner should be advised to restrain the suspected animals and to meet the diagnostician at the farm entrance with transportation if necessary and water for disinfection.

b. A diagnostician considered to be not contaminated will be dispatched to the farm to investigate.

c. The diagnostician should park his vehicle outside the farm entrance.

d. The diagnostician should change from street clothes to protective clothing (coveralls, rubber boots, rubber raincoat, rubber pants, rubber gloves, and rubber hat) at the farm entrance. Street clothes are not to be worn or carried onto the farm. Diagnosticians performing investigations are authorized to dispense with the use of rubber coats and rubber pants in hot weather and substitute lightweight cotton coveralls. However, before leaving the premises, the coveralls must be soaked in a permitted disinfectant. The use of all the rest of the rubber apparel is mandatory when investigations are being conducted and the rubber apparel must be properly disinfected after use.

e. The diagnostician should either brush or sponge a permitted disinfectant on the outside of all protective clothing before entering the farm. The disinfectant should be left at the farm entrance to be used upon departure.

f. Only essential equipment such as pencil, paper, thermometer, and, at night, a flashlight, should be carried onto the farm. If additional equipment is needed, it may be necessary for the diagnostician to return to the farm entrance, disinfect, and then go to the vehicle for it. Professional judgment may dictate other equipment to be carried initially.

g. The diagnostician should observe the suspect animal (or animals) quietly for a few minutes, then take the animal's temperature and record it. This is a good time to obtain information regarding the owner's observations.

h. The diagnostician should examine the muzzle, oral cavity, each foot, and the entire udder for lesions. He should record a description of all lesions (location, size, ruptured or unruptured, and age of lesion). Tissue specimens must be collected and submitted for examination in accordance with Section III, Parts A and B.

i. The diagnostician should observe other animals on the farm and examine those that look suspicious to determine an approximate incidence of infection. Total number of animals on farm should be recorded by species.

j. If in the opinion of the diagnostician the diagnosis is negative, he should complete items 1 through 7 on the Telephone Report and telephone the results to the Field Unit Inspection and Diagnosis Officer. (Telephone report form is given at the end of this part.)

k. If in the opinion of the diagnostician the diagnosis is positive, he should follow the Guideline for Diagnostician on a Foot-and-Mouth Disease Infected Premises (par. 2 this Part).

l. If the diagnostician is undecided as to a diagnosis, he may ask for consultation with another diagnostician or request laboratory assistance.

2. Guideline for a Diagnostician on a Foot-and-Mouth Disease Infected Premises

The diagnostician on the premises infected with foot-and-mouth disease should—

a. Issue quarantine.

b. Fill out telephone report form items 1-7, inclusive, and telephone the results to the Field Unit Inspection and Diagnosis Officer. After consultation with the Field Unit Inspection and Diagnosis Officer, the investigating diagnostician will call the State EADEO Inspection and Diagnosis Officer and transmit items 1-7 to him. It will be the responsibility of the State EADEO Inspection and Diagnosis Officer to confirm the case or assign another veterinarian for consultation or request specimens be shipped to a laboratory for confirmation. Confirmation may be based on clinical evidence, if the disease has been confirmed on a premises within a 10-mile radius, or if there is evidence of contact with a previously confirmed case. Laboratory confirmation may be required in other cases.

The State EADEO Inspection and Diagnosis Officer will assign a case number to each confirmed case. This number should then appear on all documents relating to that case. Numbers assigned to each confirmed case will be a 5-digit numerical code. The first two digits will identify the State according to the National Uniform Eartagging Plan, and the last three digits will be assigned consecutively as herds become involved. For example, 91-001, 91-002, and so on, for the State of Washington.

Herds depopulated as a result of direct contact with a confirmed case and not showing any clinical evidence themselves will be assigned a case number preceded by the initials "CH" (for contact herd). For example, CH 91-004, CH 91-005. For Additional information on coding, see the Daily Report in Section III, Part K, par. 5).

After receiving confirmation of his case, the diagnostician will call the Field Unit Inspection and Diagnosis Officer and report confirmation to him. Eradication procedures will be immediately initiated by the Field Unit headquarters.

c. Take the following action while waiting for arrival of the appraisal team, depopulation crew, supplies, etc.:

- Appraise infected animals by agreement between the owner and the diagnostician. These animals should be euthanized as soon as possible and carcasses should be soaked with approved disinfectant.
- Review thoroughly the layout of the farm with the owner, lock or otherwise secure all gates, and start making plans of operation. Consider factors such as need to build corral or fences; need to move animals; the amount of feed or hay which may have to be destroyed.
- Review plans for depopulation and disposal with owner. Make sure the disposal area is the best possible in terms of moving the animals, moving heavy equipment, possible contamination of domestic water wells, or any danger from fire, smoke, and odors.
- Anticipate needs for decontamination equipment to allow personnel, trucks, and heavy equipment to leave the premises when they are finished.
- Determine need for rodent and vector control and advise field unit to obtain services of exterminator.

d. The diagnostician should initiate operation as soon as personnel and equipment arrive. The following is the order of preference:

- Establish guard at farm entrance.
- Appraise animals and materials.
- Start trench or pit digging or fire line as soon as equipment and personnel arrive. The object is to complete the trench or fire line to coincide with the completion of appraisal.
- Start euthanizing as soon as appraisal forms are signed by owner. Observe the euthanasia operation at all times to insure that humane methods are always used. Check each animal to be sure it is dead.
- As soon as the cleaning and disinfection team arrives the members should plug all drains and soak all the barns, sheds, and milk parlor with a permitted disinfectant before starting to clean.
- The appraisal team should appraise all contaminated feed, hay, straw, and buildings to be destroyed, before leaving the farm.
- Select site for manure disposal (compost, burn, or bury).
- If milk is in bulk tanks or cooler unit, appraise with owner and arrange to acidify before disposal.

3. Check List for Cleaning and Disinfection of an Infected Premises

a. Initiate cleanup of all manure, debris, loose straw, and feed. Bury portions that cannot be burned.

b. Establish a system for hauling manure to previously selected site for disposal. This may involve several tons and may require considerable time.

c. Stalls, barns, and stanchions that cannot be cleaned out with tractors must be forked out and scraped.

d. Assign one man to heat water for making sodium carbonate (soda ash) solution (water must be 95° F. before sodium carbonate will go into solution). It may be necessary to build a temporary fire pit of old blocks, bricks, or rocks. Fifty-gallon drums cut in half make excellent containers for heating water. The sodium carbonate may be added to the water as it is being heated.

e. A third crew should be started on cleaning and disinfecting. They should start on the first area cleaned out using wire brushes, scrapers, brooms, and ample disinfectant to clean each brick, board, pipe, door, and wall. All evidence of manure or contamination should be removed prior to final disinfection.

f. Extreme care should be used in cleaning the milk parlor. The owner should be encouraged to assist and supervise this operation to prevent damage to equipment. This is an extremely important area since possibly infected animals were milked the day of the diagnosis and virus from lesions (teat, feet, and mouth) as well as virus excreted in milk will have seeded the area thoroughly. Special care should be given to all rubber equipment. It is best to burn such equipment and replace with new equipment later. Don't forget the inside of milk lines and the milk tank. Refer to Section III, Part I–Cleaning and Disinfection, par. 3, h, for instructions on C&D of milk equipment.

g. Continually check each team to assure thorough cleaning. Do not depend upon the disinfectant to do the job. Watch for stray pieces of equipment hanging on the walls or laying in window sills or in the corner of a stall. Items that are worthless should be gathered in one area for disposal. Items the owner considers valuable should be cleaned and disinfected. He should be discouraged from trying to salvage ropes, halters, and other items of little value that are difficult to disinfect.

h. Possibly contaminated hay or straw should have been appraised, removed, and buried or burned during the disposal operation. Remaining feedstuffs should be sprayed with 4 percent formaldehyde as recommended in Section III, Part I–Cleaning and Disinfection, par. 3,g, Hay and Crops.

i. Determine the need for insect or rodent control and request services from Field Unit Headquarters.

j. Clean and disinfect owner's trucks, tractors, and cars.

4. Infected Premises Security

Maintain around-the-clock security on an infected premises for the period necessary to accomplish appraisal, depopulation, disposal, and a complete soaking of contaminated areas with a permitted disinfectant.

The following is the procedure for security:

a. Close and lock or secure all entrances except one strategically located entrance to the premises. Post quarantine and warning signs at all entrances.

b. Post a guard at the one open entrance to the premises and any additional guards around the premises as deemed necessary.

c. Allow no one to enter the premises except necessary workmen and regulatory personnel wearing proper protective clothing.

d. Allow no one to leave the premises without adequate disinfection under supervision. Emergency movement from the infected premises can be arranged through the officer in charge of the infected premises.

e. Provide proper equipment for cleaning and disinfection of personal and heavy equipment off the premises (foot bath, high-pressure spray rig, brush, sponge).

f. Take all precautions to prevent the spread of disease by drainage.

g. Confine spreader animals, such as dogs and cats. It may be necessary to institute extermination measures, such as shooting, poisoning, or trapping to control predatory animals, rats, birds, and other wildlife.

h. Instruct guards to stop movements of milk, meat, eggs, bones, wool, hides, hay, straw, feed sacks, manure, or any other product capable of transmitting the infection.

i. Arrange for spraying with a permitted disinfectant the surfaces of roads or lanes that might have become contaminated outside the infected premises.

j. Guards may be removed from the premises when depopulation and disposal have been completed and the contaminated portions of the premises have been thoroughly soaked with a permitted disinfectant.

k. When the guards have been removed from the infected premises, security will be the responsibility of the cleaning and disinfection team chief.

5. Telephone Report Form

The form to use in reporting a case over the telephone follows.

Telephone Report

Veterinarian assigned to case _____ Field Unit _____
(Signature)

Telephone No. at infected premises _____ Case No. _____

Date reported _____

By Whom _____

(1) Name and address of owner _____

_____

(2) Address of infected premises _____

Map reference or coordinate _____

(3) Date of investigation _____

(4) Total livestock on the premises: Cattle _____ ; Swine _____ ; Goats _____

Sheep _____ ; Horses _____ ; Other _____

Number of purebred animals included above: Cattle _____ ; Swine _____

Sheep _____ ; Goats _____

(5) Suspect animals (number and species) _____

(6) a. History, symptoms, and temperature of suspect animals: _____

_____

_____

_____

b. Lesions:

Mouth _____ Age of lesions _____

Foot _____ Age of lesions _____

Teats _____ Age of lesions _____

Post Mortem _____

(7) Tentative diagnosis _____

_____

(8) Specimens to be submitted to a laboratory: Yes _____ No _____

Flight No. _____ Courier _____

(9) Quarantine date _____

(10) Depopulation authority given for following number of animals:

Cattle _____ Goats _____

Sheep _____ Other _____

Swine _____

(11) Depopulation crew needed: Yes _____ No _____

Number of men needed _____ Date and time _____

Method of depopulation: Firearms _____ Drugs _____ Other _____

(12) Method of disposal: Bury _____ Burn _____ Other _____

Portable corral needed: Yes _____ No _____ Date & time _____

Type of equipment needed for disposal _____ Date & time _____

(13) Appraisal team needed: Date and time _____

Purebred appraiser needed: Date and time _____

(14) Address and location of owner's other premises _____

_____

(15) Are there direct contact animals on adjacent premises: Yes _____ No _____

Owner of direct contact animals _____

Location of direct contact animals _____

(16) List movements of animals *onto* premises during previous 21 days _____

_____

(17) List movements *off* premises during previous 21 days _____

_____

(18) Indicate visits by owner or employees to other premises where animals are kept _____

(19) Give address and location of premises where employees keep livestock _____

_____

(20) Give dates of visits and address of visitors to infected premises during previous 21 days. Example: stock owners, feed salesmen and feed trucks, artificial inseminators, veterinarians, stock dealers and buyers, or other persons connected with livestock _____

_____

_____

_____

_____

(21) Possible source of the disease (owner's opinion and veterinarian's opinion) _____

(22) Garbage fed: Yes _____ No _____ Raw _____ Cooked _____

Source of garbage _____

(23) Cleaning and disinfection crew: No. of men needed _____

Date and time needed _____

(24) Cleaning and disinfection equipment needed at farm entrance _____

Date and time _____

(25) Tick and louse control equipment needed: Yes ___ No ___
(for vector borne emergency diseases)

Type of equipment needed _____ Date and Time _____

(26) Rodent control needed: Yes _____ No _____ Date & Time _____

SECTION XI—SURVIVAL OF VIRUSES

Survival of African Horsesickness Virus

Location	Period of Survival	Conditions
In insects		
Aedes aegypti	5-6 weeks	After engorgement
Anopheles stephensi	15-22 days	After engorgement
Culex pipiens	15-22 days	After engorgement
Culicoides spp.	Period not known	----
Other Media		
10% serum saline	5-6 months	39° F.; pH 7.3
Virus suspensions	Long periods	Under 131° F.; pH 6-10
Inoxalate phenol glycerine	Many years	Ambient Temperature
Horse meat, milk	Period not known	----
Fetal blood, urine	Period not known	

Survival of African Swine Fever Virus

Location	Period of Survival	Conditions[1]
Blood defibrinated	140 days	AT, stored in dark
	428 days	32° F., stored in dark
Blood, OCG	536 days	AT
	1,024 days	34-39° F.
	6 years	32° F., stored in dark
Serum, unfiltered	106 days	AT, decomposed
filtered	428 days	32° F., stored in dark
Blood on wood, bricks	Less than 70 days	AT
Blood, decomposed	5 days	AT, collected from carcass at death, stored
	128 days	AT, stored
Blood, lyophilized	Over 7 years	39° F.
Blood, in soil	112 days	AT, summer
	190 days	AT, winter
Carcass meat (boned)	Less than 188 days	39° F., stored
Bone marrow	188 days	39° F.
Spleen	280 days	AT, summer, buried in soil
	366 days	AT, winter, buried in soil
Urine	4 hours	AT, in carcass
	60 days, less than 80 days	39° F., stored
Lake water	50 days, less than 123 days (summer), 175 days (winter)	AT, bottled, buried in soil

See footnote at end of list.

Location	Period of Survival	Contitions[1]
Sunlight exposure	3 hours	AT
Feces	16 hours, less than 64 hours	AT, collected 16 and 64 hours after death
	11 days	AT, collected at death and stored
	150 days, less than 215 days	39° F., stored
Pen floors (hard)	Several weeks	AT

Survival of Foot-and-Mouth Disease Virus

Location	Period of Survival	Conditions[1]
Inside barns	15-28 days	AT, summer
On walls, etc.	35-68 days	AT, winter
Outside buildings	9-15 days	AT, summer
on walls, etc.	52-79 days	AT, winter
Walls, plaster	27 days	AT, summer
Brick	14 days	AT, summer
Abattoir waste	3 days	68° F.
Sewage	Over 100 days	36-45° F.
Fresh water	At least 30 days	AT, about 34° F.
Salt water	Over 100 days	AT, summer
Manure, liquid	39 days	AT, autumn open tank
	16 hours	closed tank
Manure, solid	29-33 days	AT, summer
	156-168 days	AT, winter
	6-9 days	Depth 30 cm. in pit
Garden soil	25-30 days	AT, summer
Soil, surface	6-7 days	AT, summer
	136-146 days	AT, winter
Corrals	345 days	AT, one instance (Calif.)
Barn mud	70 days	AT, summer
Dry sand, deep	11 days	AT
,'surface	2-3 days	AT
Hay on surface	1-10 days	AT
Hay, inside stack	30 days	AT, summer
	185-200 days	AT, winter
Hay, fodder	56-105 days	AT
Barn, fodder	140 days	AT
Straw, flour meal	5-49 days	AT
In pasture plants	1-7 days	AT, summer
	52 days	AT, winter
Mountain pastures	26 days	AT, summer
	258 days	AT, winter

See footnote at end of list.

Location	Period of Survival	Conditions[1]
Animal Products		
Blood, citrated	5 days	98.6° F.
	10 days	AT
Clothing		
Gum boots	102 days	AT
Cotton cloth	63-68 days	AT
Silk, linen	3-14 days	AT
Leather (shoes)	30-35 days	AT
Dried Blood		
On glass, brick, wood	2-3 days	AT
In meat wrappers	45 days	AT
Hides		
Green	90 days	59° F.
	352 days	39° F.
Dried	42 days	68° F.
Salted	46 days	AT
Cowhair (live)	28-42 days	AT, winter
Milk and Milk products		
Milk, whole fresh untreated	25 hours	AT
	12 days	41° F.
Milk, skim	30 hours	AT
Butter, unsalted	8 days	AT, after precooling
	26 hours	AT, no precooling
Butter, salted	9 days	AT, after precooling
	4 days	AT, no precooling
Cream, butter	45 days, even when rancid	AT
Buttermilk, skim milk, etc.	less than 20 hours, viability depends on pH (acidity)	AT, pH is about 6 or less
Cheese	5-22 hours depending -- on the amount of souring, heating	
Dried milk powder		
Moisture<6%	2 years	AT
<7%	1½ years	AT
Milk, dried on wood	2 days	AT
Meat Products		
Pork flesh	4-6 days	AT
Kidney	10 days	AT
Bovine Carcass meat	73 days	39° F.
	194 days	32+° F.

See footnote at end of list.

Location	Period of Survival	Conditions[1]
Saliva	1 day (not 2 days)	98.6° F.
	24 days (not 35 days)	73° F.
	35 days	41° F.
Urine, bovine	5 hours	AT, pH 6.8-7.6

Survival of Fowl Plague Virus

Location	Period of Survival	Conditions[1]
In bone marrow	303 days	32° F., stored
In flesh	287 days	32° F., stored
In carcasses	180 days	39° F., stored
On chicken down	240 days	AT, shade
On feathers	18 days	AT, stored
In crop contents	300 days	AT, stored
In chicken house	42 days	AT, shade
In embryonic fluid	120 days	39° F.
	20-60 days	AT, stored
In blood tissues	several weeks	AT
In insects	3-6 days	AT, after bite
In sunlight	Few minutes	

Survival of Rinderpest Virus

Location	Period of Survival	Conditions[1]
Bovine blood	Half-life 36 hours	AT, stored
Bovine blood	Half-life 2-3 days	45° F.
Bovine blood	Half-life 21 hours	98.6° F.
Lymph glands	Half-life 6½ hours	AT, stored
Lymph glands	Half-life 2-3 days	45° F., stored
Lymph glands	Half-life 1-3/4 hrs	98.6° F., stored
Thin layer bovine blood	Half-life 2 hours	93° F., unknown thickness
Dried virus	Half-life 1 week	AT, stored
Dried virus	Half-life 2½ months	39° F., stored
Lymph glands	Less than 10 days	AT, stored
Lymph glands	Less than 3 days	98.6° F., stored
Lymph glands	Less than 56 days	45° F., stored
Spleen	Over 3 years	-13° F., stored
Buried carcass	Over 2 months	Temp. unknown
In water	18-22 days	39° F., stored
In soil	3 days	AT
In discharges (Saliva, lacrimac, nasal)	3-4 days	AT, shade Box stall

See footnote at end of list.

Location	Period of Survival	Conditions[1]
Saliva, lacrimac, nasal	1-2 hours	AT, sunlight
Meat, milk, etc.	Very short when putrefaction, souring, sunlight	AT

Spread by fomites rare, usually infected animal
Optimum pH for survival = 7.0

[1] AT = ambient temperature

UNITED STATES DEPARTMENT OF AGRICULTURE
ANIMAL AND PLANT HEALTH INSPECTION SERVICE
VETERINARY SERVICES
FEDERAL CENTER BUILDING
HYATTSVILLE, MARYLAND 20782

November 14, 1972

Subject: Change in Emergency Foreign Animal Disease Eradication Guide *

To: Emergency Programs Staff
Veterinarians in Charge
Foreign Animal Disease Diagnosticians

The Foreign Animal Disease Eradication Guide should be changed as follows:

Page 60, Section 7

Delete the last sentence which reads, "Probang O-P fluids may be omitted when 5 grams of vesicular covering and fluids are available from unruptured vesicles.

Replace with -- "Probang (O-P) fluids should be submitted in all cases, even though accompanied by voluminous vesicle harvest from the same animal."

Each Foreign Animal Disease Diagnostician should have probangs of three sizes and be familiar with their use. Please advise us if additional equipment or training is required.

/s/ R. E. OMOHUNDRO

R. E. Omohundro
Director
Emergency Programs

*Xerox copy retyped for clarity.

APPENDIX 3

HISTORY AND SUBMISSION OF PROPER FORMS AND REPORTS, GENERAL, AND FOR DISEASES OF MAMMALS (from the "Red Book")

The forms included in the present edition of the "Red Book" are undergoing revision and may be superseded at the time you receive this manual. These forms are included for training purposes and to illustrate the principles involved in preparing adequate histories and reports. In event of an actual outbreak secure current forms from APHIS or your state veterinary authorities.

SECTION III–ERADICATION PROCEDURES

Part A–Procedures for Investigating a Suspected Emergency Animal Disease

1. Initial Report and Investigation

When a suspected foreign or other emergency animal disease is reported the State and Federal veterinarians in charge will jointly conduct or have conducted by their representatives a prompt field investigation. If, in their judgement, the condition found is clearly not in the nature of an emergency animal disease, a specially trained diagnostician need not be called. However, a full written report of the case should be furnished the Animal Health Division. This report should include (1) the name and address of the owner, (2) the name and address of the person initially reporting the disease, (3) the location of the premises, (4) the species and number of the animals or poultry on the premises, (5) the species and number of animals or poultry affected, (6) a description of the symptoms, including losses, if any, and (7) conclusions reached as to the cause and the nature of the condition. Form ANH 7-9 will be used to report all suspected emergency animal disease investigations.

2. Request for Investigation by a Foreign Animal Disease Diagnostician

When the initial investigation indicates that a condition may be an emergency disease, the nearest specially trained foreign animal disease diagnostician (from the standpoint of travel time) should be promptly called by telephone and his assistance requested. The diagnostician should be contacted in the most expeditious manner possible, either through his field station or directly. Any diagnostician contacted directly should advise his veterinarian in charge or other responsible official at his home station of his proposed assignment. If there is any problem in securing the services of a diagnostician, the appropriate Assistant Director or the Emergency Animal Disease Staff may be contacted for assistance.

Immediately after the diagnostician is contacted, the veterinarian in charge of the field station should also communicate, by telephone, with the appropriate Assistant Director or the Senior Staff Veterinarian for Emergency Animal Diseases and give him the history, symptoms, and circumstances leading to the action he has taken, the name of the diagnostician he has called for assistance, and the measures initiated by the State. Until the possibility of an emergency livestock or poultry disease has been eliminated, the appropriate State office should restrict movements to or from the premises involved.

3. List of Foreign Animal Disease Diagnosticians

Names, addresses, and telephone numbers of all specially trained diagnosticians, Assistant Directors and Emergency Animal Diseases Staff personnel are listed in the Consumer and Marketing Services's Directory of Consumer Protection Programs Establishments, Circuits and Officials (formerly C&MS Working Reference). (A current issue of the Directory should be readily available at all times.)

4. Foreign Animal Disease Diagnostician's Responsibilities

Upon request, the Foreign Animal Disease Diagnostician wili proceed as rapidly as possible to the designated premises and investigate the suspected disease condition. The investigation will be conducted in accordance with Section III, Part B–Diagnosis of a Suspected Emergency Animal Disease. The diagnostician will keep the Veterinarian in Charge, State Officials, and the appropriate Assistant Director informed daily (or more often if appropriate) by telephone as the investigation progresses.

5. Procedures When an Emergency Animal Disease Is Strongly Suspected

If the investigation by the Foreign Animal Disease Diagnostician discloses a suspected emergency disease of livestock or poultry, the Veterinarian in Charge, State Officials, and the Director of the ANH Division shall decide whether to (1) submit specimen material to a designated laboratory, (2) assign a specialist to the case, or (3) inoculate diagnostic animals. The diagnostician should instruct the owner to confine the affected animals until a diagnosis is made. When the condition is highly suspicious of being an emergency animal disease, the Veterinarian in Charge will consult with State livestock sanitary officials and then contact the appropriate ANH Assistant Director, or member of the Emergency Animal Diseases Staff relative to placing into effect items 6 through 15 of the following Checklist for Handling Suspected Emergency Animal Diseases.

6. Checklist for Handling Suspected Emergency Animal Disease–For use by State Veterinarian and Federal Veterinarian in Charge.

(1) Foreign Animal Disease Diagnostician requested to conduct investigation.

_____ _____

Date and Time By Whom

(2) Hold order or quarantine placed on premises by veterinarian initially investigating the suspected condition.

_____ _____

Date and Time By Whom

(3) Report of investigation telephoned to the appropriate ANH Assistant Director.

_____ _____

Date and Time By Whom

(4) Diagnostic specimens taken by courier to Plum Island Animal Disease Laboratory on Instructions of the ANH Division.

_____ _____

Date and Time By Whom

(5) Initiate epidemiological investigation: Check the livestock, people, feed, and vehicle movements onto and off the premises for 3 weeks before the appearance of the disease; also check sale of milk and other animal products. Determine wildlife status in the area.

_____ _____

Date and Time By Whom

When an emergency animal disease is diagnosed clinically, the following preparatory actions should be taken by the State and Federal Veterinarians in Charge pending results of diagnostic tests:

(6) Alert State EADEO

_____ _____

Date and Time By Whom

(7) Arrange for veterinarians to inspect herds in surrounding area.

_____ _____
Date and Time By Whom

(8) Arrange for quarantine and guard personnel as needed to handle the situation.

_____ _____
Date and Time By Whom

(9) Arrange for appraisers.

_____ _____
Date and Time By Whom

(10) Arrange for disinfection supplies and equipment.

_____ _____
Date and Time By Whom

(11) Arrange for guns and ammunition or other materials for depopulation.

_____ _____
Date and Time By Whom

(12) Arrange for excavation equipment.

_____ _____
Date and Time By Whom

(13) Define area to be quarantined on a map.

_____ _____
Date and Time By Whom

(14) Notify State police or other law enforcement personnel.

_____ _____
Date and Time By Whom

(15) Notify other States involved.

_____ _____
Date and Time By Whom

When confirmation of the pending diagnosis has been received, and the Declaration of an Emergency has been signed, eradication procedures will be initiated.

7. Procedures When an Emergency Animal Disease Is Mildly or Slightly Suspected

When the diagnostician is only mildly or slightly suspicious of a foreign animal disease, such as when only one animal is showing typical lesions and symptoms or several animals are showing atypical lesions and symptoms, the following actions should be initiated as deemed appropriate.

a. Ask for consultation with another veterinarian familiar with the disease.

b. Place "Hold Order" or "Quarantine" on premises.

c. Examine the herd several times each day for additional animals to develop symptoms.

d. Send diagnostic specimens to the laboratory.

e. Allow milk to be removed from the premises on permit only to plants offering approved milk processing.

8. Reporting Guideline

a. The initial report of an emergency disease will be accomplished using the following guide:

(1) The initial report of a suspicious disease condition will be received from the field (veterinary practitioner, county agent, owners, etc.) by the State Veterinarian or the ANH Veterinarian in Charge.

(2) State Veterinarian and the ANH Veterinarian in Charge will have State or Federal area veterinarian investigate the report.

(3) Area Veterinarian will report on the investigation to State Veterinarian and ANH Veterinarian in Charge.

(4) If in the opinion of the State Veterinarian and the ANH Veterinarian in Charge the case is of an emergency disease nature, they will request a specially trained Foreign Animal Disease Diagnostician to investigate the case.

(5) ANH Veterinarian in Charge will report suspected case to the appropriate ANH Assistant Director or Senior Staff Veterinarian for Emergency Animal Diseases.

(6) Diagnostician will report the results of his investigation to ANH Veterinarian in Charge of the State in which the case is located.

(7) ANH Veterinarian in Charge will report the results of the diagnostician's investigation to the appropriate ANH Assistant Director. ANH Assistant Director may request a direct telephone report from the diagnostician.

b. The following guide will be used in reporting emergency disease cases during actual eradication operations.

(1) Diagnostician will report items (1) through (7) in the Telephone Report to the Field Unit inspection and diagnosis officer. (See Part M, Infected Premises, Paragraph 5.)

(2) After consultation with Field Unit inspection and diagnosis officer, the diagnostician will report items (1) through (7) in the Telephone Report to the State EADEO inspection and diagnosis officer, who will confirm or deny the case and assign the case number.

(3) The State EADEO inspection and diagnosis officer will report the case to the State EADEO Emergency Operations Veterinary Officer who will call and report the case to the appropriate Assistant Director or other designated representatives at the National EADEO.

USDA-ARS

FIELD REPORT OF ANIMAL DISEASE INVESTIGATION

1. NAME AND TITLE OF DIAGNOSTICIAN	2. DATE OF REPORT	3. DATE OBSERVED BY OWNER	4. DATE OF INVESTIGATION	5. DATE FIRST REPORTED TO ADE, WASHINGTON

6A. NAME AND MAILING ADDRESS OF OWNER OF ANIMALS	6B. NAME AND MAILING ADDRESS OF OWNER OF PREMISES *(Complete if different than 6A.)*

7. LOCATION OF PREMISES	A. STATE	B. COUNTY	C. TOWNSHIP	D. SEC.	E. DIRECTION AND DISTANCE *(miles)* FROM NEAREST CITY OR TOWN	8. REPORTED BY *(Name of person)*

HISTORY AND EXAMINATION OF ANIMALS

KIND	ANIMALS: NUMBER A	AFFECTED: NUMBER B	AFFECTED: AGE C	AFFECTED: TEMPERATURE D	NUMBER: DEAD E	NUMBER: RECOVERED F	LESIONS: MOUTH VESICULAR YES G	MOUTH VESICULAR NO H	MOUTH NECROTIC YES I	MOUTH NECROTIC NO J	FOOT VESICULAR YES K	FOOT VESICULAR NO L	FOOT NECROTIC YES M	FOOT NECROTIC NO N	UDDER YES O	UDDER NO P	AGE OF LESIONS Q
9. HORSES																	
10. CATTLE																	
11. SWINE																	
12. OTHER *(Specify)*																	

13. OTHER LESIONS *(Describe)*	14. TENTATIVE DIAGNOSIS

TEST ANIMAL INOCULATIONS

15. SOURCE OF TEST ANIMALS	16. ANIMAL SPECIES AND NUMBER
17. SOURCE AND PREPARATION OF INOCULATION MATERIAL	**18. METHODS AND TIME OF INOCULATION**

19. PERIOD AND FREQUENCY OF OBSERVATIONS	20. RESULTS OF INOCULATIONS-DIAGNOSIS	21. TYPE OF FEED

LABORATORY SAMPLES

ANIMAL	SERUM A	TISSUE *(Describe)* B	SOURCE: TEST ANIMALS C	SOURCE: INFECTED OR EXPOSED ANIMALS D	DATE TAKEN E	DATE SUBMITTED F	REMARKS G	To Be Completed By Laboratory: DATE RECEIVED H	To Be Completed By Laboratory: RESULTS OF TEST I
22. HORSES									
23. CATTLE									
24. SWINE									
25. OTHER *(Specify)*									

ADE Form 7-9
Dec 1959

PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE.

COPY DISTRIBUTION–Original to Chief Staff Officer, Emergency Diseases
Copy with serum or tissue sample for laboratory diagnosis
Copy to State Official, State of Origin
Copy to Federal Official, State of Origin
Copy to Issuing Inspector

DATA ON ORIGIN OF INFECTION

26A. SOURCE OF AFFECTED ANIMALS

26B. DATE ACQUIRED

26C. TRANSPORTATION HISTORY

27A. SOURCE OF OTHER RECENTLY ACQUIRED ANIMALS

27B. DATE ACQUIRED

27C. TRANSPORTATION HISTORY

28. HISTORY OF POSSIBLE SOURCES OF INFECTION, INCLUDING OTHER FARMS OR CONTAMINATED FEED, IN THE LOCAL AREA

DISTRIBUTION DATA FOR DISEASE CONTROL

29. HISTORY OF ALL ANIMAL BY-PRODUCTS, FEED, LITTER OR OTHER MATERIAL *(which may be contaminated)* MOVED FROM THE FARM WITHIN A THREE WEEK PERIOD PRIOR TO THE INITIAL SYMPTOMS

30. POSSIBLE DISTRIBUTION BY INSECT VECTORS OR ANY OTHER MEANS *(i.e., adjoining farms and pasture, traffic-human, vehicles, predatory animals and birds, rodents, etc.)*

DISEASE CONTROL - DISINFECTION - QUARANTINE

31. OUTLINE DISINFECTION MEASURES USED AND LIST QUARANTINES ESTABLISHED

SIGNATURE AND TITLE OF ISSUING INSPECTOR

APPENDIX 4

HISTORY AND FORMS: DISEASES OF POULTRY

At the time of publication, these forms were undergoing revision and may now be superseded. They are included here for training purposes. In event of an outbreak, secure the current forms from APHIS, or your state veterinary authorities.

FORM APPROVED; BUDGET BUREAU NO. 40-R3474

U. S. DEPARTMENT OF AGRICULTURE
AGRICULTURAL RESEARCH SERVICE
ANIMAL HEALTH DIVISION

INVESTIGATION OF POULTRY DISEASES

INSTRUCTION: Prepare a separate report for each premise. Submit an original to the ANH Field Station. Use additional sheets, if necessary, and refer to item number.

1. DATE OF INVESTIGATION
2. STATE
3. NAME AND ADDRESS OF OWNER OF FLOCK *(Include Zip Code)*
4. REASON FOR INVESTIGATION — SUSPECTED ENDEMIC DISEASE ☐ SUSPECTED EMERGENCY DISEASE ☐ OTHER *(Specify)*
5. LOCATION OF FLOCK *(Town, county and State)*
6. SPECIES — TURKEYS ☐ CHICKENS ☐ OTHER *(Specify)*
7. TYPE OF FLOCK — MEAT TYPE ☐ BREEDERS ☐ PRODUCTION LAYING CHICKENS ☐
8. NAME OF PERSON AT LOCATION GIVING INFORMATION
9. OUTBREAK REPORTED TO AUTHORITY BY *(Name and address, include Zip Code)*
10. TELEPHONE NO.
11. DATE REPORTED

FIELD INFORMATION *(Also complete item 38 thru 40 on reverse)*

12. DESCRIBE SYMPTOMS EVIDENT DURING THIS INVESTIGATION
13. TENTATIVE FIELD DIAGNOSIS

14. ADDITIONS OF BIRDS TO FLOCK DURING THE PAST 21 DAYS

NUMBER A	DATE ACQUIRED B	AGE ACQUIRED C	ACQUIRED FROM *(Name and address, include Zip Code)* D

15. REMOVAL OF BIRDS FROM FLOCK DURING THE PAST 21 DAYS

NUMBER A	DATE REMOVED B	AGE C	NAME AND ADDRESS *(Include Zip Code)* D

BIOLOGIC INFORMATION

16. VACCINES USED IN FLOCK UNDER INVESTIGATION

NAME OF DISEASE A	METHOD OF APPLICATION B	BRAND OF VACCINE NAME C	BRAND OF VACCINE LOT NO. D	DATE USED E	NO. BIRDS VACCINATED F	REASON FOR MEDICATION G

LABORATORY INFORMATION

17. NAME AND LOCATION OF LOCAL LABORATORY *(Include Zip Code)*
18. PHONE NO.
19. NAME AND ADDRESS OF PATHOLOGIST AT LOCAL LABORATORY *(Include Zip Code)*
20. PHONE NO.
21. DATE SPECIMEN SENT TO LOCAL LABORATORY
22. TYPE SPECIMEN SUBMITTED — TISSUE ☐ BLOOD ☐ SPECIFY *(Liver, spleen, etc.)*
23. TENTATIVE DIAGNOSIS BY LOCAL LABORATORY
24. FINAL DIAGNOSIS BY LOCAL LABORATORY
25. TREATMENT RECOMMENDED BY LOCAL LABORATORY
26. CHECK IF LOCAL LABORATORY REPORT IS ATTACHED *(Attach if available)* ☐
27. SPECIMEN TO USDA LABORATORY FOR TESTS? YES ☐ NO ☐
28. DATE SUBMITTED
29. TYPE SPECIMEN SUBMITTED — TISSUE ☐ BLOOD ☐
30. USDA LAB DIAGNOSIS
31. QUARANTINED? YES ☐ NO ☐
32. DISPOSITION OF DEAD BIRDS — BURNED ☐ BURIED ☐ TO RENDERING CO. ☐
OTHER *(Specify)*
33. NAME AND ADDRESS OF RENDERING CO. *(Include Zip Code)*
34. DATE OF CLEANING AND DISINFECTING
35. CLEANING AND DISINFECTING SUPERVISED BY *(Name)*
36. SIGNATURE OF INVESTIGATOR
37. TITLE

ANH FORM 9-1
FEB 1966

REPLACES ADE FORM 9-1, FEB 1958, WHICH IS OBSOLETE

(Over)

FIELD INFORMATION

38. MAKE A DIAGRAM OF BUILDINGS, ROADS, FEED STORAGE, EGG ROOM, ETC. *(Code buildings, etc. by letter or No. for reference)*

39. GIVE THE FOLLOWING FOR EACH BUILDING LOCATION FOR PERIOD OF ILLNESS

DATE OF INFORMATION — A	BLDG. CODE IN ITEM 38 — B	TOTAL NO. BIRDS IN EACH BLDG. — C	AGE OF BIRDS — D	PERCENT OF MORBIDITY — E	PERCENT OF MORTALITY — F	EGG PRODUCTION *(No. Doz.)* — G
				%	%	
				%	%	
				%	%	
				%	%	

40. LAST FEED RECEIVED

A. DATE REC'D	B. BRAND OF FEED	C. NAME AND ADDRESS OF TRANSPORTER *(Include Zip Code)*

APPENDIX 5

HISTORY AND FORMS: LABORATORY REQUEST/REPORT AND RELATED FORMS

At the time of publication, these forms were undergoing revision and may now be superseded. These are included here for training purposes. In event of an outbreak, secure the current forms from APHIS, or your state veterinary authorities.

U. S. DEPARTMENT OF AGRICULTURE
AGRICULTURAL RESEARCH SERVICE
ANIMAL HEALTH DIVISION

LABORATORY REQUEST/REPORT

Direct inquiries to reporting laboratory and refer to case number.

1. NAME AND ADDRESS OF OWNER *(also NPIP Form 29 serial number, if applicable)*

2. REFERRED BY

3. TYPE OF SPECIMEN AND PRESERVATION
A. ORGANS

B. OTHER

4. REFERRAL NO.

5. DATE SUBMITTED

6. SPECIES

7. SEX

8. AGE

9. DISEASE SUSPECTED AND/OR EXAMINATION REQUESTED

10. HISTORY INCLUDING SIZE OF HERD OR FLOCK, NUMBER SICK, NUMBER DEAD. *(Use supplemental sheets if necessary)*

FOR LABORATORY USE ONLY

11. CONDITION OF SPECIMEN

12. DATE RECEIVED

13. DATE REPORTED

14. CASE NUMBER(S)

15. LABORATORY FINDINGS

16. DISTRIBUTION

17. FOR

18. BY

ANH FORM 10-1
JUNE 1966

REPLACES ADE FORM 10-1, OCT. 1962, WHICH WILL BE USED UNTIL EXHAUSTED

U.S. DEPARTMENT OF AGRICULTURE
AGRICULTURAL RESEARCH SERVICE
ANIMAL DISEASE ERADICATION DIVISION

INSPECTOR'S REPORT OF EMERGENCY ANIMAL DISEASES

SECTION A – INSPECTION, DIAGNOSIS AND QUARANTINE

1. NAME AND MAILING ADDRESS OF OWNER OF ANIMALS *(No. and street, or R.F.D. No., city, P.O. zone No., and state*

2. NAME AND MAILING ADDRESS OF OWNER OF PREMISES (COMPLETE IF DIFFERENT THAN ITEM 1. IF "SAME" SO INDICATE)

3. LOCATION OF INFECTED PREMISES			
A. STATE	B. COUNTY	C. TOWNSHIP	D. SEC

4. DIRECTION AND DISTANCE (MILES) FROM NEAREST CITY OR TOWN

ANIMALS ON PREMISES

KIND	TOTAL A	INFECTED B	EXPOSED C	QUARANTINE D
5. CATTLE				
6. SHEEP				
7. GOATS				
8. SWINE				
9.				

10. NAME OF DISEASE	11. FIRST INSPECTION DATE
12. DIAGNOSIS DATE	13. QUARANTINE DATE
14 A. TITLE	14 B. REPORT DATE
14 C. SIGNATURE OF INSPECTOR	

SECTION B – APPRAISAL AND SLAUGHTER OF ANIMALS AND DISINFECTION OF PREMISES

KIND OF ANIMAL	APPRAISAL OF ANIMALS			ANIMALS SLAUGHTERED	
	NUMBER A	VALUE B	DATE C	NUMBER D	DATE E
15. CATTLE					
16. SHEEP					
17. GOATS					
18. SWINE					
19.					

21. NAME AND TITLE OF FEDERAL EMPLOYEE SUPERVISING SLAUGHTER AND DISPOSAL.

22. NAME AND TITLE OF PERSON SUPERVISING DISINFECTION OF PREMISES

23. A. DATE DISINFECTION COMPLETED	23 B. VALUE OF PROPERTY DESTROYED BY DISINFECTION

20. ANIMALS APPRAISED BY

A. NAME AND TITLE OF STATE EMPLOYEE

B. NAME AND TITLE OF FEDERAL EMPLOYEE

24 A. TITLE OF INSPECTOR	24 B. REPORT DATE
24 C. SIGNATURE OF INSPECTOR	

SECTION C – TEST ANIMALS PLACED

TEST ANIMALS PLACED		
KIND	NUMBER A	DATE B
25. CATTLE		
26. SHEEP		
27. GOATS		
28. SWINE		
29.		

30. REMARKS

31 A. TITLE OF INSPECTOR	31 B. REPORT DATE
31 C. SIGNATURE OF INSPECTOR	

SECTION D – OBSERVATION OF TEST ANIMALS AND RELEASE OF QUARANTINE

32. REMARKS

33. DATE OF FINAL OBSERVATION OF TEST ANIMALS	34. QUARANTINE RELEASE DATE	35 A. SIGNATURE AND TITLE OF INSPECTOR	35 B. REPORT DATE

ADE Form 12-1
Oct. 1959

REPLACES IQ FORMS 2 AND 3 WHICH ARE OBSOLETE

ADE STATION COPY

STATE AGENCY COPY

WASHINGTON ADE COPY

ISSUING INSPECTOR'S COPY

VALID ONLY FOR DESTINATION STATED

U. S. DEPARTMENT OF AGRICULTURE AGRICULTURAL RESEARCH SERVICE ANIMAL DISEASE ERADICATION DIVISION PERMIT FOR MOVEMENT OF ANIMALS FOR IMMEDIATE SLAUGHTER	MOVEMENT AUTHORIZED AS FOLLOWS 1A. NAME OF OWNER OF ANIMALS 1B. ADDRESS OF OWNER (NO. & STREET OR R. F. D. NO., CITY, P. O. ZONE & STATE)

2A. KIND OF ANIMAL	2B. NO. OF ANIMALS	3. ORIGIN OF SHIPMENT			
		A. STATE	B. COUNTY	C. TOWNSHIP	D. SECTION
		4. CONSIGNED TO (NAME OF PERSON OR COMPANY)			
		5. DESTINATION (NO. AND STREET, OR R. F. D. NO., CITY, P. O. ZONE NO. & STATE)			

I certify that I have inspected the animals described above and find them to be apparently free of disease.

Permission is granted for their movement in accordance with the requirements of the State and Federal regulations and subject to permission of State authorities at destination.

6A. TITLE	6B. SIGNATURE	6C. DATE ISSUED

ADE FORM 12-3
OCT. 1960

RAIL SHIPMENTS—THIS COPY SHALL BE DELIVERED TO CARRIER AND ACCOMPANY WAYBILL.
TRUCK SHIPMENTS—THIS COPY SHALL ACCOMPANY SHIPMENT AND BE DELIVERED TO CONSIGNEE.

MAIL THIS COPY TO STATE OFFICIAL AT STATE OF DESTINATION.

MAIL THIS COPY TO STATE OFFICIAL IN STATE OF ORIGIN.

TO BE MAILED TO AND RETAINED BY FIELD OFFICE.

COPY TO BE RETAINED BY ISSUING INSPECTOR.

APPENDIX 6

STANDARDS FOR DIAGNOSIS OF HOG CHOLERA: EXCERPTS FROM AUTHORITATIVE SOURCES.

The status of hog cholera (HC) is now undergoing rapid change. If the eradication program continues with its present success, HC may become an exotic disease in the USA. It has been several years since the classic form with high mortality and morbidity has been widely observed in the USA. Milder and latent forms have resulted in diagnostic problems. For these reasons, excerpts from recent authoritative papers and protocols regarding the diagnosis of HC are included in this manual.

DIAGNOSIS OF HOG CHOLERA (HC)*

At the time of preparation of this manual (Feb. 1975), approximately a year had elapsed since the last case of HC was diagnosed. It is possible that HC may be regarded as a foreign animal disease in the near future. Since it has been an important domestic disease of swine, a great deal of information on HC is now available in texts and references. Nevertheless, differential diagnosis is now complicated in both the USA and elsewhere by changes in the nature of the disease. A much larger proportion of mild and latent cases rather than the classic fulmonating disease now characterize most outbreaks. In fact, many of the HC diagnoses effected in the course of the latter stages of the eradication program were made on the basis of routine samples taken from herds in which HC was not suspected. For these reasons brief excerpts from authoritative sources on the diagnosis of HC are included in this manual.

I. RECOMMENDED MINIMUM STANDARDS FOR DETECTING HOG CHOLERA VIRAL ANTIGEN BY THE FLUORESCENT ANTIBODY (FA) TISSUE SECTION TECHNIQUE (1)

Specimen Collection and Shipment

Tonsil, spleen and cervical lymph nodes are the tissues of choice in most instances when chronic HC is suspected ;two to three inches of the terminal ileum should also be collected. When dead or sick animals are not available for necropsy, tonsil biopsies should be collected. Tonsil biopsies are ideal for this procedure. When other tissues cannot be obtained, one inch ear tips may be submitted. Additional tissues such as kidney, lung, adrenal gland and other lymph nodes may be collected. Each piece of tissue should be placed in separate plastic bags and identified.

---

* Prepared by D. H. Ferris

Tissues should be preserved by refrigeration if the tissue can be processed within eight hours after collection. If longer time is required the tissues should be preserved by freezing. Frozen coolant cans provide adequate cooling for refrigerating specimens in transit, but dry ice is necessary for shipping frozen specimens.

Procedure for Conducting Test

A. Trim specimens so the tissue blocks are approximately 1/8 inch thick. These blocks are frozen on a freezing microtome chuck using OCT compound* or water to adhere the tissue to the chuck and to form a supporting matrix collar around the tissues.

B. Cut frozen sections eight microns thick. Tissue sections should be mounted directly on glass slides from the freezing microtome blade. Frosted or plain glass slides may be used, however, all frosted slides have the advantage that the tissue sections are less likely to wash off during subsequent processing.

C. Mounted tissue sections are immediately fixed 10 minutes in reagent grade acetone at room temperature.

D. Fixed sections are flooded with anti HC conjugate and incubated 30 minutes in a moist chamber. Conjugate should cover the entire tissue section. An applicator stick can be used to spread the conjugate over the section.

A simple moist chamber can be made from a 25 x 100 centimeter petri dish by placing two short pieces of an applicator stick and a moist ball of cotton on the bottom of the dish. The slide is placed on the piece of applicator stick to prevent capillary attraction adhering the slide to the petri dish.

*Ames Company, Division Miles Laboratory, Inc., Elkhart, Indiana.

E. Pour off the anti HC conjugate remaining on the slide and wash thoroughly in buffered saline. Excellent results have been obtained by washing actively 10 minutes through three changes of carbonate-bicarbonate buffered saline, pH 9. Clean glassware and buffer should be used for each case to make sure cells are not inadvertently transferred from one slide to another during washing. Carbonate-bicarbonate buffered saline may be prepared as follows:[1]

Preparation of Carbonate Buffer

Solution A - Na_2CO_3 53 gm.
Distilled water to make 1000 ml.

Solution B - $NaHCO_3$ 42 gm.
Distilled water to make 1000 ml.

Theoretically, a pH of 9.0 should result from mixing 44 ml. of Solution A with 1000 ml. of Solution B, but experience has shown 800 ml. of Solution B (4.2% $NaHCO_3$) and 215 ml. of Solution A (5.3%Na_2CO_3) usually produce a buffer of approximately pH 9.0. The final pH should be checked on a meter and adjusted to pH 9.0.

Preparation of Carbonate-Buffered Saline

Carbonate-bicarbonate Buffer pH 9.0 1000 ml.
Sodium cholride 8.5 gm.

Keep buffered saline tightly stoppered when not in use. Prepare small amounts frequently rather than large amounts infrequently.

F. Rinse the slides quickly in distilled water. This step is necessary to remove buffer salts that would otherwise dry on the slide.

G. Apply coverslip using buffered glycerine to hold the coverslip over the section. (Buffered glycerine is prepared by combining 9 parts glycerine and 1 part carbonate-bicarbonate buffered saline pH 9).

Controls

The ever present possibility of unidentified fluorescing material in the tissue section and the possibility of degenerating cells absorbing some of the fluorchrome dye necessitates control sections. Two additional control sections should be cut and fixed in acetone.

Control sections are processed in the same manner as the test sections except control section is flooded with equal parts of HC antiserum and conjugate and the other is flooded with equal parts of normal porcine serum and conjugate. The antiserum or normal serum should be mixed with the conjugate before the solution floods the tissue section. This mixing can be carried out in separate glassware or on the slide adjacent to the tissue section. Subsequent incubation, washing, and cover-slipping are conducted in the same manner as for test section.

It is not necessary to prepare controls from each piece of tissue processed. Routinely control sections are only prepared from tonsilar tissue. However, if tissue other than tonsil such as spleen or lymph node are found to be positive and tonsil is negative, controls should be carried out on the positive tissue.

Known HC positive section should be processed with each case. This serves as a control for the procedure and conjugate as well as the microscope. Tissue sections known to be negative for HC antigen should be processed any time it appears the fluorchrome in the conjugate may be producing undue nonspecific staining.

Interpretation

Typical fluorescing cells containing HC antigen appear light green or apple green with the fluorescence confined to the cytoplasm. Usually several

positive cells occur together to form plaques and individual positive cells are scattered throughout the tissue section. Occasionally only plaques or individual positive cells are observed.

Sections treated with undiluted conjugate have the greatest amount of fluorescence. Fluorescence is usually slightly reduced in the section treated with conjugate plus normal porcine serum. In the section treated with conjugate plus unconjugated anti HC serum fluorescence should be completely blocked or significantly less than that seen in the section treated with normal serum plus conjugate.

In actual practice the most troublesome cases are those in which small focal areas in the tissue section exhibit dull fluorescence, but neither of the control sections exhibit any fluorescence. The cause for this reaction is unknown. However, it may be due to fluorchrome staining degenerating cells. Cases of this type may be interpreted as suspicious of the HC, but the term "positive" should be reserved for cases in which unconjugated anti HC serum reduces or blocks fluorescence.

Microscopic Examination

Darkfield condenser and ultraviolet illumination are necessary. The eye pieces should be 10X. The objectives should be 10X and 25X. The condenser should be high dry darkfield. Oil immersion objective and condenser are not necessary, but may be used according to individual preference. The light source should be a HBO-200 mercury vapor lamp or equivalent. An OG 12 exciter and a blue absorbing barrier filter or the equivalent are satisfactory. An incandescent light source should be utilized to enable switching back and forth from UV to incandescent light. By utilizing both light sources in this manner it can be determined if fluorescence observed is due to light refractive unidentified fluorescing material in the tissue section. Nonspecific fluorescing

material is frequently light refractive under incandescence light whereas fluorescence due to conjugated antibodies attaching to viral antigen completely disappers under incandescence light.

II. HOG CHOLERA FLUORESCENT ANTIBODY SERUM NEUTRALIZATION TEST (2)

1. Stock virus is propagated in PK-15 cells and stored at -70°C.

2. Stock virus is titrated in PK-15 monolayers in Leighton tubes. The virus dilution used in the serum neutralization (SN) test should produce an average of 5-6 fluorescent plaques per field when 0.1 ml of virus dilution is inoculated into a Leighton tube culture.

3. Serums to be tested are heat inactivated at 56°C for 30 minutes.

4. Serums may be pooled and tested at one dilution. If pool is postivive, serums in pool maybe tested individually at one dilution or titrated at initial dilutions of 1:2, 1:8, 1:32, 1:128, 1:512.

All virus and serum dilutions are made in Earle's medium containing 0.5% lactalbumin hydrolydate and 25 millimoles $MgCl_2 \cdot 6H_2O$ (5.07 gm/liter).

5. To 1.5 ml of each serum dilution, an equal volume of virus dilution (to give 5-6 plaques /0.1 ml) is added.

6. The serum-virus mixtures are incubated at 37°C for one hour. The following control mixtures are also incubated:

a) virus dilution + hyperimmune serum
b) virus dilution + negative serum
c) virus dilution alone

7. 0.2 ml of each of the above mixtures are inoculated into a Leighton tube culture of a monolayer of PK-15 cells.

8. The inoculated cultures are incubated for 18-24 hours at 37°C.

9. The cover slip cultures are removed, rinsed, fixed in acetone and stained for 30 minutes at 37°C with HC conjugate. The slips are then

rinsed, mounted in glycerol-PBS and read in the fluorescence microscope.

10. A serum dilution is considered positive when there is a 90-100% reduction in fluorescent plaques as compared to the negative serum control.

III. HOG CHOLERA DIAGNOSTIC GUIDE

This is a guide to assist the diagnostician. No single herd or animal should be expected to demonstrate all of the following criteria for diagnosis. Final determination should be based on a composite of information gained through an orderly and comprehensive diagnostic procedure; if a category is applicable, enter the assigned points as the score and check each item observed in determining the final diagnosis. If a category is not applicable, enter "0" as the score. In all cases the professional judgement of the diagnostician is the final determining factor.

1. HISTORY COMPATIBLE WITH HOG CHOLERA (Points assigned - 20) SCORE /____/

__/Sickness in swine not vaccinated against HC.
__/Failure of antibiotic therapy.
__/High morbidity and mortality; few recoveries in unvaccinated swine.
__/Sickness not necessarily confined to any particular age group of unvaccinated swine.
__/One or more pigs dying about time sickness spreads to remainder of herd.
__/Recent additions to herd with additions, or unvaccinated swine exposed to additions, sickening first.
__/Exposure to outside infection through people, equipment, vehicles or animals.
__/Hog cholera outbreaks in neighborhood.
__/Feeding raw or improperly cooked garbage.
__/Improper use of live virus with the vaccinated swine, or unvaccinated swine exposed to those vaccinated, sickening first.

2. CLINICAL SIGNS OF HOG CHOLERA (Points assigned - 10) /____/

__/Temperatures of most of visibly sick swine 105° - 107°. (Temperatures of 10% or 10 swine, whichever is less, of the herd should be taken, including many of the visibly sick.)
__/Sick swine off feed.

2. (Continued)

__/Sick swine having eye discharge, gummed eyelids, or conjunctivitis.
__/A few (often 1 or 2) young sick swine exhibit central nervous system disturbances. (Rarely, older swine exhibit convulsions. The clinician may have to rely on other observers noting convulsions.)
__/Sick swine show purplish discoloration of skin, particularly on abdomen, legs, or ears (may not be apparent on dark skin).
__/When moved from nest, many sick swine: move reluctantly __/ exhibit weaving gait, particularly in hind quarters __/ evidence constipation followed by diarrhea __/ stand with head and tail drooped __/
__/When at rest, sick swine may either:
__/Pile up, even in hot weather. (This is usually seen fairly early in the course of the disease.)
__/Lie alone. (This is usually seen in terminal stages of the disease.)

3. PETECHIAE OR ECCHYMOTIC HEMORRHAGES (Points assinged - 20) /____/

__/Petechiae or ecchymotic hemorrhages are most consistently observed in the following: larynx __/ epiglottis __/ kidney __/ urinary bladder __/

4. PERIPHERAL HEMORRHAGE OF LYMPH NODES (Points assigned - 20) /____/

__/This type of hemorrhage assumes greater significance when it appears in nodes of more than one body region. Hemorrhages observed in some nodes may be so severe as to appear diffuse rather than peripheral. As a minimum, the following nodes should be examined: submaxillary __/ cervical __/ hepatic __/ mesenteric __/ renal __/ suprainguinal __/

5. INFARCTS (Points assigned - 20) /____/

__/Spleen-Infarcts are most significant in the spleen.
__/Colon or Cecum-Characteristic "button ulcers" are not always confined to the area near the ileo-cecal valve, but may be found in adjacent areas of colon or cecum.
__/Gall Bladder-Infarcts of the gall bladder may appear to be hemorrhages.
__/Tonsil-Tonsillar infarcts often appear as small abscesses. In HC, the tonsil may also show purplish discoloration.

6. RIB CHANGES (Points assigned - 10) /____/

__/These changes occur at the epiphyseal area of the junction of the rib and cartilage near the sternum, and are most pronounced in the sixth to eighth ribs. Abnormal calcification at the costo-chomdral junction results in a widened or irregular epiphysis, hemorrhagic epiphyseal line, or (in chronic cases) a tranverse line of very dense bone between normal bone layers just proximal to the epiphysis.

7. LEUCOPENIA DEMONSTRATED (Points assigned - 30) /____/

__/White blood cell counts should be made from acutely sick swine over six weeks of age. To demonstrate leucopenia, one or more counts should be less than 10,000/ml. The possibility of leucopenia cannot be eliminated until counts are made from at least six such swine with all ranging over 10,000/ml.

8. BRAIN HISTOPATHOLOGY POSITIVE (Points assigned - 50) /____/

__/A laboratory function involving histopathological search of brain tissue for characteristic vascular lesions.

9. FLUORESCENT ANTIBODY TEST POSITIVE (Points assigned - 70) /____/
__/Tissue Culture
__/Tissue Section

10. ANIMAL INOCULATION POSITIVE (Points assigned - 70) /____/

__/This procedure is not utilized for routine diagnosis, but is reserved for those occasions where diagnosis cannot be made otherwise, or when it is essential that initial diagnosis be further confirmed.

11. TOTAL SCORE /____/

GUIDE FOR HOG CHOLERA DIAGNOSIS

POSITIVE - 100 or more Points - In areas engaged in final eradication procedures, a positive diagnosis must always include at least 50 points in items 1 through 6 and one or more positive findings in items 7 through 10.

SUSPICIOUS - 20 - 90 Points - Further work should be done to establish the presence or absence of HC.
10 or more Points - In areas engaged in final eradication procedures, a score of 10 or more should be regarded as suspicious.

NEGATIVE - 0 - 10 Points

IV. INTERNATIONALLY RECOGNIZED PROCEDURES FOR THE DIAGNOSIS OF HOG CHOLERA (4)

1. Clinical data	Observations	Suspicion	Confirmation	Delay
a. epizootology	Contagiosity Subjects of all ages	+		
b. Symptoms	Fever Cutaneous lesions Cough-diarrhea Neurological disturbances	++		
c. Macroscopic lesions	Disseminated hemorrhages or necroses (skin-lymph nodes-heart-lungs-intestines-kidneys-bladder)	+++	C	
2. Laboratory investigations:				
d. Microsc. exam.	Material Nervous system* (non-purulent panencephalitis)	+++		48 hrs.
e. Immunofluorescence			C	
Cryostat	Tonsils *			2 to 3 hrs.
Cell culture	Spleen, mes. ganglia *			24 to 72 hrs.
f. END test **	idem		C	8 days
g. Rabbit inoc.	Spleen *	+++		15 days
h. Agar precip.	Pancreas *		C	24 to 48 hrs.
i. CF test	Liver-spleen *		C	24 hrs.
j. Antibodies		+ or C		
SN (PAV-1 strain)	Convalescent serum*			8 days
SN (HEIC test) ***				8 days
FA tests		+		2 to 3 hrs.
Agar Precip.				24 to 48 hrs.
k. hyperimmune pig inoc.	Spleen *	(differentiation from ASF)		8 to 12 days

* Material to be removed for transmission to the laboratory where the entire cadaver can not be sent.

C Confirmation of HC

** Exhaltation of Newcastle Disease virus test.

*** Test employing "ADL" cell culture adapted strain of HC virus (Nat. Vet. Lab. Kokubunje, Tokyo, Japan).

References

(1) Excerpts from: Bedell, D., McDaniel, H. A., Clark, C. D., Grey, A. P., Aikin, J. 1968. RECOMMENDED MINIMUM STANDARDS FOR DETECTING HOG CHOLERA VIRAL ANTIGEN BY THE FA TISSUE SECTION TECHNIQUE U.S. Livestock Sanitary Assoc. Proceedings, 72nd Annual Meeting. (For official USDA use).

(2) Excerpts from: Smithies, L. K. 1975 Senior Virologist, Wisconsin Animal Health Laboratory and USDA State-Federal Cooperative Program (Modification of protocol from Carbrey, E. A., Stewart, W. C. et.. al., 1969 JAVMA 155:2201-2210)

(3) GUIDE FOR HOG CHOLERA DIAGNOSIS furnished by APHIS to HC Diagnosticians

(4) Table on HC Diagnostic Procedures adapted from Rabot, L. G. 1971. PROPERTIES OF THE VIRUS OF CLASSICAL SWINE FEVER AND DIFFERENTIAL DIAGNOSIS OF CLASSICAL AND AFRICAN SWINE FEVER. Page 48, Commission of the European Communities, Directorate-General for Agriculture.

APPENDIX 7

Bibliographic materials on exotic diseases available from the Librarian, PIADC:

Monthly bibliography on exotic animal diseases - published since 1963

African horse sickness 1806-June 1967 (#6698)
Suppl. 1 July 1967-July 1971 (#6698/1)
Suppl. 2 August 1971-July 1972 (#6698/2)

African swine fever 1921-1965 (#6380)
Suppl. 1 1966-June 1967 (#6380/1)
Suppl. 2 July 1967-July 1971 (#6380/2)
Suppl. 3 August 1971-July 1972 (#6380/3)

Bluetongue in cattle (Ibaraki) 1970-1971 (#8590)

Borna disease 1926-1971 (#8675)

Cont. bovine pleuropneumonia and Mycoplasma mycoides var. mycoides
1852-1968; appendix 1969-April 1970 (#8053)
Suppl. 1 May 1970-April 1971 (#8053/1)
Suppl. 2 May 1971-April 1972 (#8053/2)
Suppl. 3 May 1972-April 1973 (#8053/3)

East Coast fever 1925-1972 (#8700)
Ephemeral fever 1878-July 1971 (#8589)

Foot-and-mouth disease in man 1695-1965 (#6381)
Suppl. 1 1966-Sept. 1971 (#6381/1)

Louping-ill virus 1925-1972 (#8701)
Swine vesicular disease 1966-1973 (#8800)
Suppl. 1 1974 (#8800/1)

Vesicular exanthema of swine 1933-1963 (#5768)
Suppl. 1 1964-June 1971 (#5768/1)

Vesicular stomatitis 1826-1963 (#5769)
Suppl. 1 1964-1967 (#5769/1)
Suppl. 2 1968-Sept. 1971 (#5769/2)
Suppl. 3 Oct. 1971-Sept. 1972 (#5769/3)
Suppl. 4 Oct. 1972-Sept. 1973 (#5769/4)
Suppl. 5 Oct. 1973-Sept. 1974 (#5769/5)